Ecology and physiology of photosynthetic organisms in highly acid streams

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ECOLOGY AND PHYSIOLOGY OF PHOTOSYNTHETIC ORGANISMS IN HIGHLY ACID STREAMS

By

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This thesis which is entirely the result of my own work, has not been accepted for any degree, and is not being submitted concurrently in canditature for any other degree.

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8. DISCUSSION

Abstract

Two surveys of the water chemistry and photosynthetic flora were carried out in England, for waters with a pH of 3.0 or less. Of the 16 sites located, fourteen were associated with coal mining, one with a barytes mine and one with an industrial effluent. One coal mining site (Site 16) and the industrial effluent (Site 15), were found to run intermittently below pH 3.0. Samples collected from 95 10 m reaches, showed that the waters were characterized by high levels of heavy metals, silicate and sulphate and that most carried moderately large levels of phosphate and combined inorganic nitrogen. The total flora from the 16 sites consisted of 24 algal species, two mosses and two flowering plants. Of the 8 species which occurred in over 20% of the reaches sampled, *Euglena mutabilis* was the most widespread and abundant species.

One stream with a pH gradient of 2.6 - 7.0 (Brandon Pithouse Acid Stream) was studied in greater detail. Observations were made in respect to seasonal variation and to changes in chemistry and flora along the pH gradient. In addition, monthly estimations of the maximum standing crop of algae and moss protonema were carried out for one year. Analysis of these data suggests that over a large pH range, H+ concentration has the greatest influence on the number of species present in the stream; although other factors (eg. precipitation of ferric hydroxide) may also affect the presence and abundance of some algae.

In addition to field studies, laboratory experiments were conducted on five species of algae isolated from Brandon Pithouse Acid Stream. These included an examination of the effect pH had on growth and morphology and also the relationship between low pH and heavy metal toxicity.

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1. INTRODUCTION

1.1 General introduction

Although some research has been carried out on the effects of hydrogen ion concentration on aquatic organisms, many basic questions remain unanswered. Further, only a small proportion of the work which has been carried out on the low pH environment has concerned photosynthetic organisms. Some progress has been made in understanding the community structure and physiological adaptations which occur in the thermal acidic environment (Brock & Brock, 1966: Doemal & Brock, 1970: Castenholz, 1973). However, similar work has not been conducted on the non thermal acidic environment, even though the occurrence of such habitats is probably more widespread and available to biologists than the thermal springs. Most of the emphasis in the non thermal acidic field has been placed on the formation and abatement of the acidic waters where they constitute a pollution problem, and therefore, the physio-chemical and engineering aspects have received considerably more attention than the biological ones (eg. Braley, 1951 - 1954: Parsons, 1956-58: Hawley, 1971).

As mentioned in more detail in 1.2, highly acidic waters have a worldwide distribution, however, although their existence is known in England (Glover, 1971), little work has been conducted on the chemistry and biology of these waters. It was therefore considered that a study of the chemistry and flora of highly acidic waters in England should be carried out.

A general review of many aspects of low pH environments has been included because the published data relating to photosynthetic organisms are limited. The various organisms recorded are considered in order of their position in the aquatic food chain, beginning with the larger animals. Bacteria are discussed in two sections, the first (1.24) relating to their involvement in acid formation, and the second (1.8) to the distribution of species which are not necessarily involved in this.

1.2 Low pH environment and production of acid

1.21 Sources of acidity

The various types of acidity which occur, originate either from organic or inorganic sources. Many waters are acidic due to the presence of organic humic acids derived from natural decomposition of plants; however, the resulting pH values are rarely below pH 3.0 (Brock, 1969) and where they do occur, it is usually due to inorganic acid contributing towards the total H^+ concentration. Pearsall (1938), examined the pH of woodland and moorland soils and found that in the upper layers of the soil which were exposed to the air, oxidation was taking place and resulted in low pH values. The lowest values recorded were on cotton-grass moorland at pH 2.80. However, Pearsall also found that in the bog situation, the substitution of water for air prevented the oxidation reaction taking place and hence the decrease in H^+ concentration resulted in pH values around pH 3.5.

Other natural sources of acidity which often lead to values below pH 3.0 are thermal springs and volcanic lakes, such as those found in Japan. The acidity of both sources is due to the oxidation of H_2S and/or SO_2 in the volcanic gas. This oxidation process can lead to pH values of about 1.0 and probably constitutes the most extreme non-thermal acid environment.

A third source of acidity which may produce extremely low pH values is from industrial effluents. Invariably, the low pH is caused by the presence of inorganic acids in the effluent rather than organic acids. Industries involved in the production of batteries, iron smelting, wood pulping, chemical distillations, munition and some textiles may produce acids as waste products (Klein, 1957). It would appear from River Authority reports, that in this country, at least, these effluents are largely treated before being discharged into surface water. The most common source of highly acidic water is a result of mining activities often associated with coal. The principal source of the highly acidic water in this case is due to the production of sulphuric acid from the oxidation of sulphurous material. The formation of acidity is considered in detail in 1.22 and constitutes the main source of extreme acid conditions reviewed in this study.

Acid mine drainage results from the passage of water through mines (and mine spoil heaps) where iron disulphide, usually in the form of pyrites, marcasite or pyrrhotite is exposed to the oxidizing action of air, water and possibly bacteria. Such conditions are found in some lignite, pyrite, zinc, copper, gold, silver, and lead mines as well as coal mines (Temple & Koehler, 1954). The occurrence and formation of acid has been well documented, the following are examples of a large bibliography: Braley (1951 - 1954); Parsons (1956); Barnes & Clarke (1964); Brant & Moulton (1960); Glover (1967); Hanna et al. (1963); Boyer (1972).

As a result of the many possible sources of sulphuric acid, highly acidic conditions are likely to occur where mining has exposed pyrites. Such conditions have been reported in many countries, including North America (eg. Parsons, 1956; Kinney, 1964; Boyer 1972), Australia (Blesing, 1974) New Zealand (Kaplan, 1956), South Africa (Harrison et al., 1958 - 1962) and several European countries including Britain (Glover, 1967), Denmark (Dahl, 1963) and Czechoslovakia (Fott, 1956).

The acid mine drainage from bituminous coal mines in North America presents a major pollution problem and has been the subject of many general surveys which have been conducted in an attempt to ascertain the chemical reactions involved in the acid production (Braley 1951 - 1954; Hanna et al., 1965).

1.22 Formation of acid mine drainage

The pyritic minerals are normally chemically stable in the reducing conditions of undisturbed strata, but oxidize slowly to form soluble sulphate compounds when exposed to the atmosphere. This reaction can and does occur naturally, where outcropping coal seams containing pyrites become exposed to the air by natural erosion. The occurrence of acid mine drainage, often obvious by its characteristic yellow - orange colour, led to the discovery of coal in the United States 270 years ago (Eavenson, 1942). The production of acid is not, therefore, entirely due to man's operations, but to the normal oxidation process between oxygen and sulphur. The mining of coal has, however, greatly increased the amount of acid produced.

As it is the exposure of pyritic material to the atmosphere and ground water that caused the production of sulphuric acid, then mining techniques can play an important role in the production and abatement of the acid. The iron sulphides are generally a waste product of mining and are exposed to the atmosphere in the shafts and in the spoil tips on the surface. Thus the treatment of the spoil and the shafts after they have been abandoned is important in the prevention of acidity. Practices such as deliberate flooding of the mine to quench a fire in the spoil or underground, are likely to increase the problem (Glover, 1967).

Unlike other forms of pollution which increase or decrease in proportion to production, acid mine drainage increases with coal production, but does not necessarily decrease with its cessation. The acid often continues to run for years after the mine has been abandoned.

The problem has increased rapidly with demand for coal, so that now a point has been reached in the major bituminous coal mining areas of America where many of the streams and rivers are unfit for industrial, domestic or recreational use, unless first treated. It has been estimated that in the United States there were over 10,000 miles of streams and 29,000 acres of impounded water which were seriously affected by acid mine drainage in 1971 (Lundgren, 1971).

1.23 Chemical reaction involved

Although the chemical reactions of pyrites oxidation have been studied in some detail, the reactions are not completely understood when considered in the context of the field situation in which they are formed. Temple & Koehler (1954), Hanna et al. (1963), and Boyer (1972) have reviewed the literature on the chemical aspects of acid mine drainage. There is general agreement that the basic chemical change is from pyritic material to iron sulphate and the subsequent oxidation to other salts proceed as follows: (adapted from Brant & Moulton (1960)

Step 1. Sulphide to Sulphate;

When sulphide is exposed to the atmosphere, it may theoretically oxidize in two ways, with water as the limiting condition. The first reaction, assumed to take place in the dry, produces an equal amount of sulphur dioxide and ferrous sulphate.

(I) $FeS_2 + 5O_2 = FeSO_4 + SO_2$

iron pyrites ferrous sulphate sulphur dioxide

However, if the oxidation process occurs in the presence of water, then the direct formation of sulphuric acid as well as ferrous sulphate will be produced.

$$\text{(II)} \quad FeS_2 + 2H_2O + 7O_2 = 2FeSO_4 + 2H_2SO_4$$

iron pyrites — ferrous sulphate — sulphuric acid

As most mines contain a certain amount of water then the latter reaction is favoured.

Step II Ferrous sulphate in the presence of sulphuric acid and oxygen is oxidized to ferric sulphate and water.

$$\text{(III)} \quad FeSO_4 + 2H_2SO_4 + O_2 = 2Fe_2(SO_4)_3 + 2H_2O$$

ferric sulphate

Step III. Precipitation of iron

The ferric sulphate combines with the hydroxyl ion of the water to form ferric hydroxide. In an acid environment ferric hydroxide is largely insoluble and precipitates out as an orange-yellow precipitate.

$$Fe_2(SO_4)_3 + 6H_2O = 2Fe(OH)_3 + 3H_2SO_4$$

ferric sulphate — ferric hydroxide — sulphuric acid

It has been suggested that the ferric ion may enter into an oxidation reduction reaction with iron sulphide, whereby the ferric back-triggers the oxidation of more sulphide to the sulphate, thus increasing the production of acid. Barnes & Clarke (1964) postulated that acid production occurred under anaerobic conditions, thus dismissing the essential role of O_2 suggested by many other workers. Clarke (1967) however concluded that the reaction could not be supported by the kinetic evidence.

The rates of reaction of pyrite (FeS_2), marcasite (FeS_2)

and pyrrhotite ($Fe_n S_{n+1}$) under acid mine drainage conditions are significantly different. Braley (1954) found that pyrrhotite reacted 18 times and marcasite 9 times the rate of pyrite.

The oxidation rates of other pyrite materials such as sphalerite and chalcopyrite are slower compared with iron sulphides. However, ferric sulphate readily attacks these sulphides according to the reaction

$$Fe_2(SO_4) + XS = XSO_4 + 2FeSO_4 + S$$

where X = Zn, Cd, Cu, Ni and Co (Blesing et al., 1974).

Other reactions which occur as a result of the low pH created by the release of sulphuric acid include the motility of Na, K, Ca, Al, Mn and Si ions. Under low pH conditions assemblages are formed, for example:

$$2KAlSi_3O_8 + H_2SO_4 + H_2O = Al_2Si_2O_5(OH)_4 + K_2SO_4 + 4SiO_2$$

At higher pH values the mobility of these ions is reduced and they precipitate out to produce yellow, orange and white sludges of various sulphates, as well as ferric sulphate. The grey-white precipitates sometimes encountered at pH values between 3.0 and 6.0 are mainly due to Al and colloidal silica (Blesing et al., 1974).

1.24 Role of bacteria

The association of bacteria with acid mine water has been noted since the earliest investigations of that environment. Powell & Parr (1919) reported that sulphur oxidation appeared to be hastened by the presence of bacteria or some catalytic agent. Davidson (1930) and Carpenter & Herndon (1937) also arrived at similar conclusions. In 1947 Colmer & Hinkle suggested that the bacterium *Thiobacillus thioxidans* was involved in the conversion of sulphur to sulphuric acid and Temple & Colmer (1951) gave the name *Thiobacillus ferrooxidans* to an iron and thiosulphate oxidizing acidophilic bacterium. Leathen *et al*. (1956) isolated *Ferrobacillus ferrooxidans* from bituminous coal mines at pH 2.0 to 4.5. They found that this chemoautotrophic bacterium could completely oxidize 200 mg l^{-1} of ferrous iron in 3 days. Ashmead (1955), in his studies of mines in Scotland, claimed that for every ton of sulphuric acid produced by chemical reactions, approximately 4 tons were produced by bacteria. Baker & Wilshire (1970), carried out a study of the role of *Ferrobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Ferrobacillus sulphooxidans* using a pilot plant. They found that the oxidation of ferrous iron and sulphide was increased, but that the bacteria did not alter the dissolution rate of pyrites.

Whilst there is much evidence that these and other species of chemoautotrophic bacteria are implicated in acid production (Walsh & Mitchell, 1972; Manning & Cooke, 1972), their detailed role is not yet fully understood. Lundgren (1971) and Singer & Stumm (1970), studied *Thiobacillus ferrooxidans* in detail and consider it essential for the rapid production of acid. These bacteria were shown to accelerate the reaction by a factor of more than 10^6 over the chemical reaction.

However, Smith & Shumate (1971), in a report concerning the rate of pyrite oxidation in the field, found that the main sites of pyritic oxidation lie above the ground water table and are only exposed to the vapour phase, not the water. They proposed that the acid forms in small droplets which are carried away by the ground water to produce an acid effluent and laboratory data show that the contribution by bacteria in these conditions is insignificant. Further evidence which supports the insignificant role of bacteria, is that the addition of bacteriocidal agents did not reduce the production of acid (Lorenz, 1962).

There appears to be a considerable amount of evidence to suggest that bacteria are implicated in the production of acid in some cases, but that the formation of acid is not entirely dependent on their presence. There is a need for the use of more field pilot schemes, such as that used by Smith & Schumate (1971), so that the many observations that have been made in the laboratory can be tested under field conditions.

1.25 Rate of reaction

The steps in the oxidation of pyritic material are apparently complex and involve several simultaneous reactions for which the kinetics are not completely understood. Many of the studies of the sulphide to sulphate oxidations made in the past, have been based on the overall reaction and not on the individual steps. Also, much of the work has been carried out in the laboratory with pure crystalline pyrites, and it is not certain how applicable the data are to the dynamic conditions of the mines.

Singer & Stumm (1970) reported that in the absence of bacteria the rate determining step was the ferrous oxidation. Kim (1968) reported that the rate of oxidation was dependent upon the ferrous concentration, temperature and amount of dissolved oxygen. Barnes & Clarke (1964) suggested that bacteria are implicated in the speed of oxidation, but that without biooxidation the chemical reactions would, in the end, produce the same amount of acid, but at a slower rate.

Silver & Lundgren (1968) suggest that the rate of ferrous oxidation is controlled by the catalytic action of *Thiobacillus ferrooxidans*. The more recent work of Smith & Shumate (1971) considers the critical reaction to be the oxidation of pyrites by oxygen and not the oxidation of ferrous to ferric iron, which they maintain does not occur at the pyrites surface. They found that the majority of acid formation takes place at the surface of the pyrites, where it is in contact with water vapour. The formation of the acid is a continuous "weeping" process, where droplets form due to the hygroscopic nature of the ferrous and sulphate ions. The rate of oxidation is therefore considered to depend on the oxygen concentration, temperature, humidity of the air and the pH.

Controversey over the role of the bacteria and the rate determining step will need careful consideration if control of the acid production is to be carried out efficiently.

It is still unknown why certain mines produce acid effluent in varying amounts when neighbouring mines in the same strata do not produce acid.

1.26 Hydrology

Ground water invariably acts as the transporting agent for most sources of acid pollution, as well as being involved in its formation. Therefore, the hydrology of the area is important when considering the production of acid.

The hydrological aspects of acid mine drainage have been considered in detail by Brant & Moulton (1960), Collier et al. (1955), Ahmad (c 1971) and Emrich & Merritt (1969). Smith & Shumate (1971) categorized the removal mechanism of the acid into the ground water. The first and least important, is the flushing action of the rise and fall of the water table, the second is the removal of the oxidation produced by water percolating down through cracks in the overburden, after heavy rainfall. The last mechanism is due to the constant weeping of the acid down the walls and has been reviewed (see 1.25), this is considered by the authors to be the most important.

Prior to mining, the rocks above the coal seams are usually completely saturated with ground water. These overlying rocks are fractured during mining and the water drains through the rocks into the shafts, which then act as conduits draining the water and acid away into the surface. The immediate effect of mining on the ground water is usually the lowering of the ground water table. In some cases, the underlying, or associated ground water, is not drained from the rocks but flows into the other associated ground water areas and thus contaminates them. The sinking of wells from one aquefer to the next, may cause the contaminated water to move down the hydraulic gradient to lower uncontaminated aquefers, thus distributing the acid pollution many miles from its source.

Although much of the acid mine drainage originates from abandoned mines which flood naturally over a period of time, acid production in the working mines is not uncommon. Water in the shaft is itself a problem to mining, but the presence of acid water can considerably increase the handling problems. The water is usually pumped out of the shafts and then diverted to the nearest ground water, thus causing considerable contamination.

1.27 Abatement of acid mine water

As previously mentioned, the primary concern of most of the research carried out in this field has been with a view to the control of acid formation. There are two possible methods of control. The first is to prevent the acid formation in the mines and the second is to treat and neutralize the acid effluent before it reaches the streams.

The methods of control at the source have been based on the principle of excluding contact between the three essential components, air, water and pyrites (Glover, 1967) Many attempts have been made to divert the water from the acid producing area and seal off abandoned shafts to reduce the air contact. In addition, attempts have been made to cover the pyrites with non acid forming material. However, because the source of acid is often so diverse, segregation methods are not very successful in the majority of mines.

The second treatment option has been used with varying degrees of success. Lime and limestone neutralization plants have been commonly used, but are very expensive when large amounts of acid water have to be treated. This process also creates a sludge of high water content and large concentrations of iron and heavy metals which present difficult and expensive disposal problems (Barnes & Romberger (1968). Other methods such as the deliberate flooding of the deaper mines has met with some degree of success, but again, results are unpredictable and this method may, in fact, enhance acid formation. The use of bacteriocides have failed to make any significant contribution to the reduction of the acid.

Glover (1967) reported favorably on the use of biochemical oxidation of ferrous salts in conjunction with limestone grit neutralization. This method could be successfully and economically used with dilute acid drainage water.

It appears that until a relatively inexpensive and efficient treatment process has been developed, the streams and rivers will continue to be polluted by these effluents. The acid mine drainage problem in Britain is similar in form, but a very small fraction of the size of the problem that is found in America. Glover (1967). However, with increasing

Table 1.1 Classification of mine drainage waters.

After Lundgren *et al.* (1971)

	Class I	ClassII	Class III	Class IV
pH	2-4.5	3.5-6.6	6.5-8.5	6.5-8.5
Acidity ($CaCO_3$)	1000-15000	0-1000	0	0
Ferrous iron, mg l^{-1}	500-10000	0-500	0	50-1000
Ferric iron, mg l^{-1}	0	0-1000	0	0
Aluminium, mg l^{-1}	0-2000	0-20	0	0
Sulphate, mg l^{-1}	1000-20000	500-10000	500-10000	500-10000

After Parsons (1964) - for lakes receiving acid water

	Type I	Type II	Type III	Type IV
	Red lakes	Transitional lakes	Blue lakes	Grey lakes
pH	1.2-2.5	2.5-3.5	3.0-4.0	above 4.0
Acidity mg l^{-1} (as sulphuric acid)	high - 5500	high - 3500	high - 300	-
Iron mg l^{-1}	> 65	130 in spring, 15 remainder of year	< 30	low
Turbidity	present all year	present in spring	none	low to normal

pressure to reduce the pollution in the surface and ground waters in Britain, the consequences of the problem become more important and treatment of acid mine drainage essential.

1.3 Physical and chemical characters of acid mine drainage

Many of the surveys of acid streams have included brief descriptions of some of the chemical and physical aspects of the water. Hawley (1971) described a typical acid mine drainage as characterized by low pH, high iron and high sulphate concentrations and abnormal heavy metal concentrations including manganese, copper, cobalt, zinc and nickel. The chemical composition of the water is dependent on the surrounding geology over which the acid flows as it is being formed. Consequently, the chemistry of the water will vary between the different geological areas. However, there is sufficient similarity between the different types of coal mine water to create four general classes, (Lundgren _et al_. 1971) of which classes 1 and 2 can be considered the most damaging (see Table 1.1 These categories are a useful guide, but they do not include information on the heavy metal concentrations, or the nutrient status of the waters. Parsons (1964) classified the acid strip mine lakes on the basis of their physical and chemical characteristics and concluded that the progressive oxidation and precipitation of iron oxides in the water was the key to classification of lakes.

Although various aspects of the chemical composition of acid water have been determined by many workers, for example Roback & Richardson (1969), Kemp (1967) and Klein (1957), (see 8.25 for further details), the report of Van Everdingen, (1969) on the acid springs in Kootenay National Park, British Columbia, remains one of the most extensive. He studied the chemical aspects of a range of sites from pH 2.5 to 5.5, measuring 11 cations, including 6 heavy metals, 6 anions, plus CO_2 and O_2 concentrations and the physical parameters pH, Eh, temperature and conductivity. The concentrations recorded agree with the broad description given above by Hawley. However no information was given on phosphorus and nitrogen.

The nutrient status of the water appears to vary considerably but generally the water can be considered as slightly to moderately eutrophic in character. Roback & Richardson (1969) and Bennett (1969) determined the nitrogen and phosphorus concentrations of several sites and found levels varying from PO_4; 4.8 - 0.5 mg l^{-1} and NO_3; 6.46 - 0.1 mg l^{-1}.

1.31 Relationship between water chemistry and species

As already stated, there have been several attempts to describe, or partly describe, the chemical characteristics of acid water, however, very few authors have attempted to relate the species composition and the chemical environment. From the observations made by Bennett (1969), Besch _et al_. (1972) and Patrick _et al_. (1974) it would appear that pH or acidity had the controlling influence on the species presence rather than other parameters such as heavy metal concentrations. However, there is some evidence from the work of Besch _et al_.

that heavy metal concentrations do play a secondary role in determining the species composition of acid waters, although this will depend upon the pH value of the water (see 8.57).

1.4 Effects of acidic water on terrestrial wild life and fish

1.41 Terrestrial wildlife

The presence of acid mine water pollution is felt not only in the aquatic environment but also in the surrounding area. The presence of acid in a major stream complex, as found in the U.S.A., may result in a dramatic reduction in the animal population of the surrounding area (Kinney, 1964). This is partly due to the continual disturbance of the surrounding countryside by the mining activities and partly because of the lack of food and cover normally provided by the vegetation in and around the acid streams. Mammals, birds and reptiles which rely on the river systems for food and breeding are particularly affected by the devastation of the normal river environment by the acid mine water. Fish predators, such as otters, bears, racoons and various species of fish eating waterfowl, are notably absent from areas where the low pH and associated factors have removed the fish population. Although data and observations are lacking in the literature, the reports by Boccardy & Spaulding (1968) and the Appalachian Regional Commission (1968) indicate that even though it is difficult to determine the specific reasons why a species is absent from an area, the effect of acid mine drainage is deletèrious to terrestrial vertebrates.

The absence of fish from these waters is of particular importance, especially in view of the increasing demand for freshwater sport fishing. Kinney (1964) estimated that in the U.S.A., 5890 miles of stream and 14967 acres of impoundment had a potential for fish and wildlife habitat, if the acid pollution was removed. The problems encountered in the U.S.A. are several degrees greater than any where else in the world; however, fish kills have been reported in other countries and potential fishing rivers are spoilt by the presence of acid waters eg. South Africa and Denmark.

1.42 Fish

There is much literature on the pH limits of fish in freshwater and this has been evaluated critically by Doudoroff & Katz (1950) and Lloyd (1968). There seems to be general agreement that fully developed fish can live between pH 5.0 and 9.0 and that below this range certain species are capable of surviving and reproducing down to pH values of 4.2, (eg. pike). Other environmental factors such as CO_2 concentrations and hardness of the water are important in determining the pH limits of the fish. High levels of CO_2 (100-200 mg l^{-1}) in the water appear to influence the toxic effect of low pH and fish kills may occur at pH values which are normally not harmful.

Although several species can survive around pH 4.0 for some time, their reproductive ability is diminished and the hatching success of the eggs is greatly reduced below pH 5.0. For salmonoid eggs, a marked reduction occurs below

4.8 although the adults have been observed at pH 4.5.

The pathological effects of low pH on fish are not completely understood. Ellis (1937) subjected goldfish to low pH and reported that death was due to suffocation by the precipitation of mucus on the gill epithelium. Lloyd & Jordan (1964) found no mucus on the gills, or apparent damage to the gill tissue of trout which had been held at pH 3.4 for 7½ hours. The authors attributed their death to acidemia.

Although the data regarding the effects of large amounts of iron on fish are limited and confused, there seems to be an indication that the lethal effects of acid mine water are increased by its presence. Even if the iron is not directly toxic, where it precipitates out it is likely that the fish population will inevitably be reduced following a reduction in the benthic community. There does not appear to be much data on the combined effects of low pH and high heavy metal concentrations. Perhaps some of the anomalies concerning the toxicity of iron may be attributed to the heavy metals.

Whatever are the reasons for the absence of fish from these waters, it is clear that restoration of the acid water to a pH in which fish can survive, ie. pH 5.0 and above, will ultimately result in an improvement in the wildlife and recreational facilities of polluted areas.

1.5 Effects of acidic waters on the fauna

1.51 Benthic fauna

Of the organisms which inhabit acid waters, the benthic and planktonic fauna have probably received the most attention and therefore a little more is known of their distribution.

Harrison and various co- workers (1958, 1960 and 1965) in studies of the fauna of S. African acid streams reported that the fauna was impoverished, but not necessarily of low density. There appears to be characteristic species which are resistant to the acid conditions; for example, Harrison _et al_. found that the populations of streams were dominated by caddis and chironomid species. The caddis _Leptocerus harrisoni_ and _Argyrobothrus_ sp. thrived at low pH, where other species of caddis were eliminated. Often several of these acid tolerant species were more abundant than in the normal streams, probably due to the lack of competition and predation in the acid environment.

Jewell (1922) and Lackey (1938, 1939) noted the fauna of acid waters. Lackey found 12 species of macroscopic invertebrates from pH 2.2 to 3.9. Of these, _Chironomum_ lavae and the midge _Caretha_ sp. were abundant. Chironomidae were also reported tolerant to low pH (2.8) by Warner (1968). He found that in water at pH 2.8 - 3.8 the maximum number of species was 12, whereas at pH 4.5 and above the number increased to 25 species. Blackflies, mayflies, and stoneflies were not found below pH 4.5. Roback & Richardson (1969) investigated the effects of mine drainage (pH 3.0 - 7.0) on aquatic insects and found that species of the Odonata, Ephemeroptera, and Plecoptera were eliminated by low pH, and that the species _Ptilostomis_ (caddis), _Sialis_ (alderfly) and _Chironomus_ *attenuatus* (Diptera) were reduced but could tolerate the high acidity.

The distribution of *Tendipes plumosus* in 19 strip mine lakes (pH 2.3 - 7.6) was studied by Harp & Campbell (1967). They reported that *T. plumosus* was the only tendipedid established at pH values below 6.0 and that although the adults were collected on the surface of the lake at pH 2.3, the pupae were unable to emerge below pH 2.8. The distribution of this species appeared to be controlled primarily by the absence of leaf detritus, not the pH value. Stockinger & Hays (1960) also recorded the genus *Tendipes* as the most numerous group, present in 3 strip mine lakes. Other major field studies into the influence of pH on benthic fauna, include Dinsmore, (1968), Patrick *et al.* (1974) and Henrick & Cairns (1972).

1.52 Laboratory studies

Several laboratory studies have also been made on the effects of acidity on aquatic insects. Stickney (1922) recorded that the nymph of the dragonfly *Libellula pulchella* was able to tolerate pH 1.0 for 12.5 hours. Bell & Nebeker (1968) conducted a study on the short term tolerance of 10 species of aquatic insect to low pH. The caddisflies, *Brachycentrus* and *Hydopsyche* were tolerant to pH 1.5 and 3.15 respectively for 96 hours, but for survival of the population over a longer period, higher pH values were required (approx. pH 3.8). Bell (1971) investigated the effect of low pH on the survival and emergence of 9 aquatic insects, including, dragonflies, stoneflies, caddisflies and mayflies. He concluded from his studies that the pH tolerance of the species varied considerably, and that caddisflies were the most tolerant and mayflies the least. Brachycentrus americanus was again the most resistant, withstanding pH 2.45 for 30 days. The emergence of the insects appeared to be the critical stage of the life cycle and required a much higher pH value for a 50% success rate. Bell recommended that to ensure a large population of insects the water should be above pH 5.5.

1.53 Zooplankton

The amount of literature discussing the zooplankton of acid stream water is considerably less than for the benthic fauna. Lackey (1938) reported several microinvertebrates present in streams of low pH. In particular, Distyla sp., Actinophrys sol, and Oxytricha sp. were well represented in the total population. Of the 3 lakes studied by Heaton (1951) the rotifer Brachionus ureolaris dominated the most acid lake (pH 2.96 - 3.3). This rotifer was also reported to be the dominant plankter in Lake Osoresan-Ko, Japan, at pH 3.0 (Uéno, 1958). At a slightly higher pH, Stockinger & Hays (1960) reported the rotifer of the genus Keratella to be the most numerous. Other genera which occurred in smaller numbers, in low pH lakes, were Cyclops, Daphnia, Diatomus and Scapholeberis.

Parsons (1968) carried out one of the most extensive studies on the effect of acid water on the fauna of streams. He studied several sites along Cedar Creek, Missouri, which were subjected to varying amounts of constant and intermittent acid pollution. From his work on the recovery of the streams, he concluded that certain planktonic and benthic animals were more tolerant and better adapted to acid conditions than others. Included among these were the rotifers *Brachionus urceolaris* and *B. havanaensis* and *Keratella quadrata*. It was evident at stations where the intermittent pH decreases occurred, that the population was dominated at all times by acid tolerant species. Although there was a decrease in the number of species in the more acidic reaches, the number of individuals was higher than in compatible non acidic reaches. The repopulation of the streams following cessation of the acid conditions was found to be a function of the length of the life cycle, eg. the planktonic species with the shorter life cycle reappeared before the benthic organisms.

1.6 Effects of acid water on photosynthetic organisms

1.61 Macrophytes

It would appear from the literature that the macrophytic flora is restricted to a few acid tolerant species. Lackey (1938), in his survey of acid streams, reported that *Typha latifolia* was the most abundant vascular plant, although *Isoetes* spp. also occurred. Patrick et al. (1974) also recorded a species of *Isoetes* present below pH 4.0, although *Vallisneria* sp. was the most dominant plant recorded.

Species of *Eleocharis* seem commonly to be associated with the acid habitat. Heaton (1951) found *Eleocharis palustris*, *Typha latifolia* and *Carex* sp. to be the only plants in a lake at pH 2.4 - 3.8. With an increase in pH to above 5.0 the plant community increased by 7 species. Bell (1956) also reported *Eleocharis obtusa* and *Typha latifolia* at pH values 3.4 and 3.0 respectively, whilst Ehrle (1960) found *Eleocharis acicularis* in Pennsylvanian streams at pH 3.0. One of the most extensive macrophyte surveys of acid waters was carried out by Moore & Clarkson (1967) and they confirmed that *E. acicularis* was the most abundant species present in the W. Virginian streams.

Harrison (1958, 1965) did not report *Eleocharis* present in the S. African acid streams he studied; however, *Typha latifolia*, *Phragmites communis* and the moss *Sphagnum truncatum* were found at pH 2.9. The species which were more abundant at higher pH values (3.7 - 4.3) were *Scirpus fluitans*, *Juncus exsertus* and *J. oxycarpus*. Harrison concluded that strong acid pollution eliminated some species, but at the same time, encouraged the acidophilic species to colonize the extreme environments.

1.62 Algae

Although algae are the basic components of the food chains in most aquatic environments and therefore of fundamental importance to the productivity of the other organisms, their reaction to acid conditions has received little attention. There have been several studies in which the algal species have been noted in passing, but very few which have dealt specifically with the algal flora and the tolerance of the individual species to the extreme environment.

The most extensive studies of the influence of acid water on the algal community, as a whole, have been carried out by Lackey (1938), Bennett (1969), Warner (1968), Steinback (1966), and Weaver and Nash (1968). The studies of Hustedt (1938), Cholnoky (1958), Jorgensen (1948), Merilainen (1967) and Patrick et al. (1968, 1974) were primarily concerned with the pH preferences of diatoms. In addition to the discussion of the available literature given below, some of these data are also summarised in a comparison of the results reported in this study, with those obtained by other workers (Table 8.2)

One of the most detailed accounts of the photosynthetic organisms in acid mine drainage, is that of Lackey for sites in Indiana and W. Virginia. He recorded 76 species of algae and protozoa over a pH range of 1.8 to 3.9. He concluded that at or below pH 3.9, the number of species found in acid water was small, the largest being 11 species at pH 2.6, while several samples showed none at all. However, where typical acid tolerant species occurred, they often did so in large numbers. The species occurring most frequently were Euglena mutabilis (85% occurrence), Chlamydomonas sp. (70%), Navicula diatoms (66%), and Ochromonas sp. (38%). Lackey considered it possible to determine the water quality by the relative abundance of a limited number of easily recognizable species.

Bennett investigated 17 stations (pH 2.69 - 7.0) in W. Virginia located on creeks, rivers and pools receiving acid drainage. He reported finding 107 species of algae at 8 stations below pH 4.1; 25 of these were restricted to the 8 acid stations but the remaining 82 were found at higher pH values. Algae characteristic of mine polluted water were those species which were common to a range of habitats. There were a few algae that were found to be consistently abundant over a period of months, these were: Euglena mutabilis Ulothrix subtilis, Pinnularia braunii, Eunotia tenella (possibly E. exigua), Ulothrix sp., Frustulia rhomboides and Penium jeneri.

Bennett also found a reduction in the number of species as the acidity increased and pH decreased and concluded, as did Lackey, that from the number of species present and their relative abundance, the range of pH and acidity could be estimated. However he added that abundance was often dependent on the algal species present and the season.

The number of species reported by Bennett was considerably larger than the numbers found by other authors. Joseph (1953) found 20 species of algae, most of which were diatoms, from 10 streams in Ohio; unfortunately he did not give the pH values recorded for the species. Weaver & Nash (1968) recorded 20 species from 6 stations on one stream of pH 3.0 - 4.0 in Kentucky. The flora of this stream was dominated by filamentous algae and Euglena spp. Of these, Euglena was found to be dominant in the more acid reaches. Warner (1968) compared two streams, one pH 2.8 - 3.8 and the other pH 4.5 and above and found that the lower pH supported 10-19 taxa of algae, while the less polluted contained 33 species. The most abundant species of the more acid areas were, Ulothrix *tenerima*, *Pinnularia termitina*, *Eunotia exigua* and *Euglena mutabilis*. Steinback (1966) also recorded *E. mutabilis* in water below pH 3.0, together with *Eunotia* spp., *Navicula* spp., *Chlamydomonas* spp. Above this pH, species of *Ulothrix*, *Microspora* and *Mougeotia* were commonly found.

In the S. African streams investigated by Harrison and his co-workers, the dominant species in a reduced flora were filamentous and diatoms. The diatoms that were often abundant below pH 5.0 were *Eunotia exigua*, *Frustulia* spp., *Pinnularia acoricola*, *P. subcapitata*, *Achnanthes microcephala* and *A. minutissima*. These species are considered by Cholnoky (1958) to be characteristic of acid conditions. Other studies of the diatom flora of acid streams include those species and therefore, they may be considered common to the low pH environment. Besch *et al.* (1972) used diatom communities as indicators of acid and high heavy metal conditions. They considered *Eunotia exigua*, *Achnanthes microcephala* and *Pinnularia interrupta* var. *beceps* as primary indicators of acid and high concentrations of Zn and Cu.

In addition to the apparently common and abundant diatoms reported, the species *Pinnularia braunii* var. *amphicephala* might also be considered as tolerant of extremely low pH conditions. Uéno (1958) reported it's presence in a Japanese lake at pH 2.7 and Satake (1974) found that it contributed considerably to the total productivity of the lake at pH 1.7.

In these extremely low pH waters, *Chlamydomonas* spp.

appear to be quite common. Fott (1964) reported _Chlamydomonas applanata_ var. _acidophila_ as growing well at pH 1.0 and Uéno (1958) and Satake (1974) also found it in a Japanese lake at pH 1.7 and 1.8. The species _Cynanidium caldarium_ was also reported present in the same Japanese lake at pH 1.8. Although _C. caldarium_ is considered a common member of the thermal acidic environment, there are no other records of this species in the non-thermal acidic habitats.

Blue-green algae are noticeable by their absence in the majority of reports on acid waters. This subject has been investigated by Brock (1973) who surveyed many acid sites but could not find any blue-green algae below pH 4.0, and only occasional occurrences below pH 5.0. These observations are supported by the majority of surveys and therefore it seems fair to conclude that the absence of blue-green algae is a characteristic of acid waters below pH 4.0.

1.7 Tolerance of photosynthetic species to low pH and heavy metals

1.71 Low pH

The majority of the literature reviewed has been concerned primarily with observational studies on the effects of low pH in the field situation. With the exception of the role of bacteria in acid formation, and _Cyanidium caldarium_ in the thermal acidic habitat, very few studies have attempted to determine the physiological aspects of an organisms tolerance to low pH. Whilst there is a need for more detailed field work, it is also necessary to determine the physiological characteristics of acid tolerance, if the whole problem of acid pollution is to be considered.

There have been several attempts to determine the pH tolerance and improve the growth of acidophilic species in culture, but very few of these species are included in the list of commonly occurring extreme acidophiles. Fott & McCarthy (1964), McCarthy *et al.* (1965) and Cassins (1974) have studied the nutrient requirements and to some extent also the pH tolerance of *Chlamydomonas applanata* var. *acidophila* and reported that it was not fastidious in its nutrient requirements and could tolerate pH values around 2.0 at a wide range of light and temperature conditions. Although it did not have any marked nutrient requirements, its growth at low pH was improved by the addition of liver fraction and increased iron concentrations (Cassins, 1974). Several other acidophilic species which are not reported commonly from extreme acid conditions, have also been studied by the authors mentioned above. These include *Carteria acidicola*, *C. turfosa* and *Chlamydomonas* spp. *Carteria acidicola* was reported by Fott & McCarthy (1964) to be similar to *Chlamydomonas acidophila* in its nutrient requirements and growth rate, where as *Carteria turfosa* was found to have a possible absolute requirement for some vitamins and was not as tolerant to lower pH values as the other species.

As mentioned previously in this section the thermophilic, acidobiont *Cyanidium caldarium* has received detailed studies in the laboratory. Doemel & Brock (1971) demonstrated that the species has a pH optimum of about 2-3 and is capable of growth at pH 1.0. Ascione _et al_. (1966) found that in a poorly buffered medium, the alga reduces the pH of its growth medium in batch culture at higher pH values.

Several species of _Euglena_ have been investigated for pH tolerance, including the apparently extreme acidophil _Euglena mutabilis_. Dach (1943) found that it could survive at pH 1.4 for 12 hours in basic medium. Kostir (1921), Jahn (1931) and Schoenborn (1950) have examined the nutrient requirements of E. _gracilis_ and _E_. _viridis_ and reported tolerance to moderately low pH. _E_. _viridis_ was also found to be capable of autotrophic growth. Moss (1973) investigated the effect of the initial pH on 35 species of algae. He found that _Euglena gracilis_ and _Eunotia exigua_ were the only species that would grow below pH 4.0.

Although not commonly associated with acid water, several species of _Chlorella_ have been tested for pH tolerance. Hopkins & Wann (1926) studied the relationship between H ions and a species of _Chlorella_, and reported pH 3.4 as the limit of its growth. Kessler (1965) conducted an extensive study of 51 autotrophic _Chlorella_ strains of 7 species and found that there were specific differences which could be used as taxonomic characters. Strains of _C_. _ellipsoida_ were the most resistant, growing at pH 2.0 - 3.0.

In addition to determining the pH limits of algal species, some effort has been made to determine the effects of pH on the availability of naturally occurring elements. Oborn (1960) and Hunter _et al_. (1950) have reviewed the role of iron in natural waters but little is known of the effects of pH on it's availability. Cassins (1974) found that at lower pH values the demand for Fe was increased.

Foy & Gerloff (1972) examined the response of _Chlorella pyrenoidosa_ to aluminium and low pH and showed that at lower pH values (4.6) there was an increased requirement for Al. They also induced tolerance to normally toxic concentrations of Al by using stress techniques.

1.72 Heavy metals and low pH

Very little work has been carried out on the combined effects of low pH and the toxicity of heavy metals. M. R. Droop (1974) suggested that the intolerance shown by many algae to low pH, is due to high levels of heavy metals, although he had no experimental proof (pers. comm.). Besch _et al_., (1972) found in the field situation that many acid tolerant species were also tolerant to high levels of heavy metals, but that acidity is the primary factor influencing species distribution in the low pH situation. Further discussion of the findings of Besch _et ai_. are given in 8.57.

1.73 Mechanisms involved in pH tolerance

Although efforts are being made to determine the nutrient requirement and resistance of organisms to pH, the basis of their tolerance remains unknown. There is some evidence in acidophilic bacteria that acid stable proteins may be involved (Doetsch _et al_., 1967, Gale & Epps, 1942).

Manning & Cook (1972) have suggested that an energy dependent system actively removes H ions from the cells. However, even if these systems are true for bacteria there is no reason why they should exist in plant cells. Cassins (1974) has suggested that specilized membranes must be involved in acid resistant algal species. Clymo (1963) demonstrated an ion exchange mechanism in Sphagnum in which H ions were released in exchange for other metal ions; he also demonstrated that the exchange rate was proportional to the amount of polyuronic acids in the cell wall. Further discussion of the possible mechanisms involved is given in Chapter 8.

1.8 Effects of acidic water on bacteria and fungi

It is now generally accepted that bacteria are associated with acid water and probably with acid formation (see 1.24). The chemoautotrophic bacteria associated with iron and sulphur oxidation appear to be indigenous to acid water. The most commonly occurring bacteria are Thiobacillus thiooxidans, T. ferrooxidans and Ferrobacillus ferrooxidans. (Leathern, 1953). Recently Walsh & Mitchell (1972) isolated an acid tolerant filamentous iron bacteria of the genus Metallogenium, and Manning & Cook (1972) found a new acidophilic member of the genus Pseudomonas, at pH 3.5.

In addition to the extensive work on these bacteria, several studies have been made on the microorganisms which are not thought to be involved with the production of acid, but are found growing in the acid environment. Joseph (1953) isolated 40 species of bacteria during a study of Chio streams of pH 2.0 - 4.0. The genera represented were Bacillus, Micrococcus, Sarcina, Escherichia, Aerobacter, Thiobacillus, Crenothrix and Microsporium. The majority of these were among the genera Bacillus and Micrococcus. Fungi were also relatively abundant and represented by seven genera: Aspergillus, Trichoderma, Helminthosporium, Alternaria, Penicillum, Trichothecium, Cladosporium. However, the numbers of both bacteria and fungi increased considerably with an increase in pH. Weaver & Nash (1968) compared two branches of a creek, one acidic (pH 3.0 - 3.5), the other did not receive acid drainage. They isolated a total of 41 genera of fungi; 17 were common to both branches and 20 were in the acid branch only.

Of the 41 species present, only 3 genera of truly aquatic fungi were represented, these were Achlya, Aphanomyces and Saprolegnia. The observations of Weaver & Nash indicated that drainage from the acid strip mine appeared to increase the fungal flora.

Tuttle et al. (1968) compared the bacterial flora of an acid and an alkaline stream, and reported that the two streams had different characteristic species. In the acidic stream the iron and sulphur oxidizing bacteria were common, whilst in the alkaline stream, they found low numbers of acid tolerant heterotrophic microorganisms.

Species of yeast have also been commonly found in acid waters. Cooke et al. (1960) isolated 3 genera of yeast, Candida, Rhodotorula and Trichosporon. Rogers & Wilson (1966) and Weaver & Nash, also reported the isolation of species of *Rhodotorula* from acid water. Ehrlich (1963) studied the microbiology of acid drainage from a copper mine and found several species of yeast, including *Rhodotorula* sp. and *Trichospora* sp. It would appear from the literature that the resistant non photosynthetic population of acid waters is quite varied and characteristic of the conditions. One remarkable example of resistance was found by Starkey and Waksman (1943) who isolated two species of acidiphilic fungi. *Acontium velatum* and a species thought to be related to *Cephalosporium* grew at between pH 2.0 and 0.3 in a saturated solution of copper sulphate.

1.9 Aims

The main aims of the study were to carry out detailed chemical and floristic surveys of acid waters of pH 3.0 and below in England. In addition, one stream was to be studied in more detail so that the seasonal variability in both the chemistry and flora might be investigated.

It was hoped that from field and laboratory observations, it would be possible to establish which factors influenced the distribution and growth of photosynthetic organisms in the low pH environment.

2. METHODS

2.1 Locating the sites

In an attempt to locate water at and below pH 3.0, two methods of approach were used. Initially, representatives of the National Coal Board and all River Authorities in England were consulted. As information available was often rather vague, every possible location was checked. Several reported low pH sites were not sampled, either because their flow was found to be intermittent, or because at the time of sampling their pH was not within the stated categories of the sampling programme (see 2.2). In addition to the direct approaches made to these Authorities, a literature search was carried out using old mining records in order to establish the whereabouts of large quantities of pyrytic material and also for the occurrence of acid mine waters. Several of the most probable sites were visited but no acid waters were found. Industrial effluents are generally less well publicised than mine drainage and therefore it is likely that there may be several which are either discharged intermittently, or run only short distances from the discharge before being diluted out.

2.2 Sampling programme

All sites sampled included flowing water, at least part of which was known to flow continuously throughout the year. Many sites consisted of one or two larger streams draining several small flushes. Invariably, the streams consist in part of excavated ditches, which were made for the purpose of diverting and controlling the flow of effluent. These streams and flushes are referred to throughout the text as stream complexes.

At several sites, pools of various sizes and depths were included in the complex (see 3.1). Although these pools were connected by streams they often consisted in part of stagnant water. Smaller, temporary pools also occurred in several complexes following long periods of dry summer weather, when the flow from the seepages was considerably reduced and evaporation occurred at a high rate.

All physical, chemical and floristic sampling of a stream complex was carried out from particular 10 m reaches. Methods of collection of physical, chemical and algal samples are given in 2.3, 2.4 and 2.5. In addition to the information collected for the sampling reaches, general observations on the topography of the stream complexes were made and are summarized in Chapter 3.

A reach was designated either at the source of the stream, at a confluence with a second stream, or below the inflow of a side flush. Every reach had its own distinct chemical compostion. Extra samples were also taken from reaches designated at points where there was an obvious change in the flora. Therefore, the number of reaches per stream complex depended on the length of the stream and the number of side flushes, or stream confluents present along its length. Where a side flush was deep enough for the physical parameters to be measured and the collection of water samples to be made, then it was classed as a separate stream.

Samples were also collected from all of the pools present in the complexes. Because of the unstable nature of the substrata it was often both difficult and dangerous to sample any distance from the shoreline. Therefore in these instances, a section of the pool delimited by a 10 m length of its perimeter was treated as a reach. Although the sampling of the pools was not completely satisfactory, it was considered that very few species were overlooked because the majority were found growing in the shallow waters near the shoreline. In the smaller, more shallow pools, an effort was made to take samples from all areas of the substrata.

Two surveys of the water and flora were carried out, the first taking from August to early October 1973 to complete and the second was done in the latter part of February 1974. These are termed late summer (A) and late winter (B) throughout the text. The number of sites, stream complexes and 10 m reaches for both surveys A and B are summarized in Table 2.1. The details of the number of reaches and pools per site are given in Table 3.1. In survey A, 13 sites were visited, of these, 12 were associated with mining, and one was an industrial effluent. At these sites 19 stream complexes were sampled from 46 distinct 10 m reaches.

Minor differences occurred between the number of reaches sampled at each survey. One coal mining site (site 2) was

Table 2.1 Summary of sampling programme(excluding industrial effluent, site 15)

	abbreviation used in text	no. sites	no. stream complexes	mo. 10m reaches
late summer survey	A	13	19	46
winter survey	B	14	20	49
total common to both surveys	-	13	19	43
total from both surveys	A+B			95

added between the two surveys and it was impossible to gain access to another stream complex on the second survey (site 4, reaches 5-7). At sites 4 and 8 some reaches of the complex were dry during the summer period, but were flowing in the late winter survey. These were therefore only sampled on one occasion making a total of 52 different 10 m reaches sampled during both surveys and 43 10 m reaches common to both surveys. With the exception of current speed, which was measured only during the second survey, all details of the field measurements and sampling procedures were identical for both surveys.

Shortly after the completion of the second survey, acid water was found at a site associated with coal mining, (site 16). However, the stream was found to run at pH 3.0 only at times of relatively low flow and was therefore classed as an intermittent site. The Industrial effluent near Minehead was also in this category, because on the return visit the pH of the water was found to be 7.2 compared with 3.0 on the previous occasions. The data recorded for these sites have been included in the table of floristic and water chemistry data, but they have not been included in the analysis of data.

2.22 Brandon Pithouse Acid Stream

In addition to the surveys, an acid stream with a pH gradient, draining Brandon Pithouse colliery, Co. Durham (site 3) was considered in more detail, and a separate programme was set up (see Table 2.2). As described in 3.3

Table 2.2

reach number	1	2	3	4	5	6	7	7b	8	9	10	11	12	13	14	15	16	17	18	19
no. times water chemistry sampled between October 1972 and July 1974	6	6	6	6	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6
no. times water chemistry sampled between July and November 1974	2		2	2	2			2	2		2	2		2	2		2			2
no. times water chemistry sampled between November 1974 and March 1975	2		2	2	2			2	2		2	2		2	2		2			2
total number of times sampled	10	6	10	10	9	5	6	10	10	6	10	10	6	10	10	6	10	6	6	10
no. times standing crop sampled	12	12	12						12		12			12	12		12			12

Sampling programme of water chemistry for Brandon Acid Stream from October 1972 to March 1975.

there are two sources of acid water draining from Brandon Pithouse, these are referred to as stream A and stream B. Monthly water and floristic samples were taken at the source of stream A from October 1972 to April 1975 (see 5.1). At approximately 3 monthly intervals during this period the whole stream complex, including stream B was sampled. The sampling procedure of the 10 m reach systems used in both surveys was also applied to the regular sampling of the Brandon Pithouse stream. The stream was initially divided into 19 10 m reaches, from the source to where Redburn enters the River Deerness (see 3.2). After a number of quarterly surveys the 19 reaches were reduced to 11. These were considered to present an adequate description of the chemical and floristic characters of the stream. In addition to the regular samples, algal samples were taken on several occasions at random from sites other than those designated as sampling reaches. These checks never produced species which were not already recorded in the monitoring system.

As a result of the changes in the pH gradient of the stream which occurred in July 1974 (see 3.5), the data are presented in three sections. These included the periods; October 1972 - July 1974, July 1974 - November 1974 and November 1974 - April 1975. The number of chemical samples taken at each reach during these periods are given in Table 2.2.

In addition to the chemical and floristic data collected for Brandon Pithouse Acid Stream, estimations of the maximum standing crop for algae were made. These measurements were also carried out on the 10 m reach basis used for chemical sampling. Nine reaches of similar physical and topographical features were chosen and sampled at monthly intervals from July 1974 - June 1975. A summary of the sampling programme is given in Table 2.2

2.3 Physical parameters

The data collected for the physical parameters recorded are displayed in Chapter 4.

2.31 Current speed

The Ott Small current flow meter, model 10.150, was used to determine current speed. The values recorded refer to the fastest current speed of the reach. Where no result is given for current in Table 4.1 this was either because the reach consisted of stagnant water, or the water was not deep enough for measurements to be taken, ie. less than the minimum working distance of 40 mm. The results are expressed as $m\ s^{-1}$.

2.32 Total discharge

Monthly measurements were made only at the source of stream A, Brandon Pithouse (site 3). In order that the total discharge could be measured, a pipe was sequered in the stream, such that the total volume of water passed through the pipe. Using a graduated collecting vessel and a stop watch accurate to 0.1 seconds, the total discharge was intercepted by the vessel until three parts full. The volume collected was then measured and the time taken to collect the volume recorded. This procedure was repeated 10 times at each sampling, and the mean value determined. Results are expressed as $l\ s^{-1}$.

2.33 pH

Measurements were recorded both in the field, using a Pye Unicam portable model No. 293, and also in the laboratory, at a temperature 15°C on an E.I.L. pH meter. Because of the acid nature of the water, the difference between the two values never exceeded 0.1 units.

2.34 Oxygen and temperature

The Lakelands Instrument portable meter with a Mackereth type electrode was used for all measurements. The results are expressed as percentage saturation and degrees centigrade respectively.

2.35 Conductivity

Laboratory measurements were made using a Lock Conductivity Bridge. Results are expressed as micromhos.

2.36 Optical density

Measurements were made directly on a Uvispek spectrophotometer at 420 nm using 400 mm silica cells.

2.37 Redox potential

Redox potential was measured using the Pye Unicam portable model No. 293. As the redox electrode was available only towards the end of the study, only a few measurements could be made.

2.4 <u>Chemical parameters</u>

2.41 Collection of water samples

Before samples were collected, all containers were soaked in 10% HCl for 24 hours and then rinsed six times in glass distilled water. This procedure was carried out to ensure removal of adsorbed trace elements, and to destroy any living cells which might be present.

Water for analysis was filtered in the field through an acid washed No.2 "Sinta" glass funnel, to remove large suspended matter and the majority of plant material. Both the filter and the collecting vessel were rinsed well with sample water before the final collection was made.

Samples for cation analysis were collected in 100 ml "Pyrex" bottles. These were used in preference to polythene because no measurable contamination of any element was found, whereas some sources of polythene are known to leach large quantities of Zn, Cu and Fe.

Samples for analysis of anions, pH redox potential and conduvtivity were collected in heavy duty polythene containers, using the same procedure as for cations.

Although the chemistry of highly acid water is likely to be reasonably stable during storage, care was taken to maintain the samples at low temperature, especially over a long distance and during hot weather. On return to the laboratory cations samples were stored at 4°C in the dark, until analysis was carried out. Careful checks were made to determine the loss of trace elements over a long period of storage. Samples for anion analysis were determined on return to the laboratory but where this was not practicable, they were stored at -10°C.

2.42 Analysis of cations

Cation analysis was carried out using a Perkin-Elmer model 403 atomic absorption spectrophotometer. The following elements were analysed: Na, K, Mg, Ca, Zn, Cu, Mn, Fe, Al Pb, Co, Ni. The standard conditions used in the Perkin-Elmer manual were used. Pb was analysed using the Ta sampling boat procedure (Kahn 1968).

2.43 Analysis of anions

The following anions were analysed: PO_4. NH_4, NO_3, Cl, Si, SO_4 and acidity. All optical density measurements involving colorimetric procedures were carried out on a Uvispek spectrophotometer (Hilger and Watts). Because of the unusually high concentrations of ions found in acid water, normal analytical techniques could not be applied in all cases. Where interference was encountered and alternative methods employed, standard additions were performed to confirm that the methods were satisfactory.

PO_4-P The normal stannous chloride procedure for phosphate determinations (American Public Health Association, 1973) gave low recovery, therefore exyraction with hexanol was used (Mackereth, 1963). In addition to removing interference the sample is also concentrated, allowing for a detection limit of 0.001 mg l^{-1} PO_4-P, as compared with 0.01 mg l^{-1} PO_4-P by the A.P.H.A. method.

NH_4-N The procedure of direct nesslerisation of the sample was adapted for analysis of NH_4-N (A.P.H.A. 1973). Slight modifications to the normal techniques were made. As most samples contained large concentrations of ions, in particular

Fe, it was necessary to clarify the samples. This was achieved by the addition of 0.5 ml of $ZnSO_4$ to 40 ml of sample, and the dropwise addition of 0.1 N NaOH to a pH of 10.5. The precipitate was centrifuged down at 3000 r.p.m. for 5 minutes and 25 ml aliquots of the supernatant was taken for nesslerization. A detection limit of 0.01 mg l^{-1} NH_4-N was achieved using this technique.

NO_3-N The high concentrations of Fe found in the acid water caused considerable interference in the colourimetric determination of NO_3-N. Attempts to remove the Fe by precipitation also reduced the level of NO_3-N in the sample. Therefore the samples were passed through a column of cation exchange resin (Amberlite IR 120 H form) prior to analysis. It was necessary to wash the exchange resin with 10% HCl and double distilled water at regular intervals. To ensure that the sample was not being diluted out as it passed through the column, 300 ml of sample was passed through and only the last 50 ml collected for analysis.

During the first survey (A) of acid streams NO_3 was determined using the method of Hammond (1959), involving 3-dimethylnaphthidine. The disadvantage of this method was the occurrence of a highly coloured blank and the occasional instability of the colour reaction, allowing a detection limit of only 0.5 mg l^{-1} NO_3-N. Therefore, the more sensitive method of Montgomery & Dymock (1962) using 2,6-xylenol was adapted for all subsequent NO_3-N analysis. It was necessary to carry out all procedures at a constant temperature of $8^{o}C$ as the reactions are temperature dependent. In particular, care was taken in the preparation of the 2,6-xylenol solution. Using 400 mm cells it was possible to attain a detection limit of 0.05 mg l^{-1} NO_3-N.

NO_2-N The presence of high levels of Fe caused interference with the colourimetric determinations of NO_2-N (Crosby 1967). However, no NO_2 could be detected after pretreatment by passage through an ion exchange column. It is possible that only trace amounts of NO_2 were present in the samples and were lost on the column. Therefore, no results are presented for NO_2-N.

Cl The argentometric titration for chloride (American Public Health Association, 1973) did not give an end point because of interference. The samples were therefore clarified by adjusting the pH to 10.5 with 1N NaOH and then re-adjusted to pH 7.0 before titration against silver nitrate.

Si The heteropoly blue method (American Public Health Association, 1973) was used for Si determination because of large concentrations of Si in acid water, samples were invariably diluted 10 times.

SO_4-S The method of Colson (1973) was employed for SO_4-S analysis. 300 ml was passed through the cation exchange resin, Amberlite IR 120 H form, and the last 50 ml collected. As high levels of SO_4 were present, many of the samples were diluted by a factor of 100 before being titrated against barium perchlorate.

Acidity The determination of acidity was carried out by hot titration to an end point of pH 8.3 using phenolphalein indicator (A.P.H.A., 1973). Potentiometric determinations to a pH of 8.3 were also carried out in conjunction with the indicator. It was necessary to dilute the majority of samples by times 100. The results are expressed as $CaCO_3$ mg l^{-1} and include any weak acids, strong acids, and acid salts that were present in the samples.

2.5 Sampling for algal and moss species

2.51 Designation of sampling area

In an effort to reduce the possibility of a species remaining undetected, each reach was sampled with extreme care. Organisms were considered part of the acid environment only when they were either submerged or emergent. Species growing on the side of the streams above the splash zone were not recorded.

A minimum of 6 x 100 mm^2 areas were collected from each reach and placed in 20 ml tubes. The samples were taken from the following areas within a reach:- areas of visually different plant growths; areas representing the various types of topography present in the reach; areas representing the different substrata; areas within the last 2 categories but where there was not obvious growth; a composite sample was taken from all physiognomic forms and different substrata with or without obvious growth. Additional algal samples were taken from the stream pools and flushes which were outside the designated reaches. This was done in an attempt to identify every species growing at the sites sampled.

In addition to the collection of samples, on site visual estimations were made of several factors connected with the samples. These included estimations of the percentage cover within a reach of those species easily recognizable macroscopically. Details of the topography, substratum size and composition, of each 100 mm^2 samples area were made. The depth and angle of substratum at which the sample was taken were also recorded. These data were used firstly in an effort to give a general description of the habitat of the species and secondly, to be included in a much larger sampling programme being carried out in the Department of Botany, Durham University. This study is being carried out with the view to determine more accurately the environmental parameters which influence the growth of algae in a wide range of aquatic habitats. As this sampling programme is, as yet, incomplete the analysis of data by computation of the acidic aspects is not included in this text.

2.52 Harvesting of algal samples

The varied habitats in which the organisms grew required different techniques for collection of the samples from the 100 mm^2 areas. On solid surfaces, not covered with mud, silt, precipitate or other debris, samples were scraped off with a knife into the tube. On surfaces coated with a friable over layer, either the sampled was 'scooped' directly into the tube or it was sucked up using a pipette. The disadvantage of the pipette method was that it tended to block, especially when the substratum included coarse sand. An improved sampling technique involved the use of an 8 mm diameter solid plastic tube attached to a large syringe. The end of the tube was placed over the sampling area and pushed carefully into the overlaying substratum, whilst drawing the sample up into the tube. The algae were then transferred to the collecting vessel. Care was taken to wash the sampler carefully between samples. Whilst the larger diameter of the tube removed the problem of blockages, the pipette was preferred where the surface was uneven.

Where mosses and emergent angiosperms were present, a sample was taken and the excess water squeezed from the material into a sample tube. This procedure was employed so as to identify any algae which were associated with the macroscopic plant material. The material was also examined for any species which was not removed by the 'squeeze' technique.

All samples were transported in a cooled thermos and stored at 8^{o}C until a microscopic study could be made.

2.53 Microscopic examination of samples

As the literature indicated that the number of species in the acidic environment was relatively small, efforts were made to ensure that all samples were examined thoroughly. The samples were viewed as soon as possible on return to the laboratory. From each 100 mm^2 sample at least four slides were prepared and studied. In addition to the identification of species, an estimation was made of the relative abundance of the species present. This was recorded on a 1 to 5 scale, and corresponds to the widely used system of scoring ie. present, occasional, frequent, abundant, very abundant. This method is highly subjective but was considered useful, especially when combined with the estimates of percentage cover (see 2.51) of a species.

Where diatoms were present, subsamples were boiled in nitric acid for approximately 20 minutes, after which hydrogen peroxide was added until the solution cleared. The samples were washed with distilled water and a permanent slide prepared using Naphrax as the mounting medium.

Species were only recorded as being present in a reach if they were alive. If dead specimens were recovered they were only considered relevant if they were in sufficient numbers as to suggest that they had been previously growing in the reach.

2.6 Estimation of standing crop of algae and moss protonema

Estimations of the maximum standing crop of algae, for 9 reaches down Brandon Pithouse stream, were made by determining the amount of chlorophyll a present in a given area. The method used was based on the principle outlined in the I.B.P. Handbook, 1974. As communities growing on sediments were to be considered and contamination by indistinguishable detritus was unavoidable, chlorophyll was selected as the parameter for the measurement of standing crop, because of its specificity to plant material. The extraction method also facilitated the analysis of a large number of samples on a regular basis. Other non radio-active techniques for estimation of standing crop presented either potential interference with elements in the environment (eg. Fe) or the impossibility of removing detritus from the sample. Patrick et al. (1974) working on acid streams in the Appalachian area, U.S.A., found that where heavy precipitate occurred, they recorded an actual loss of oxygen rather than the expected gain by photosynthesis. This was thought to be due to the presence of reducing agents in the sediment which interfered with the technique.

The main disadvantage of the chlorophyll a determination is usually associated with difficulties in harvesting the samples accurately. An additional problem is that the pigment per unit area or weight, is influenced by many environmental and internal variables, such as age of the plant and nutrient status of the water. The problems of harvesting usually encountered in 'most' streams, are reduced in many acid streams and in particular in Brandon Pithouse stream. The substrata are usually found to be clay or silt, which facilitate the removal of algae, and thus makes the determination more accurate.

2.61 Harvesting and storage of samples

As estimates were made at monthly intervals at the same 10 m reaches, it was not possible to give a complete picture of the standing crop for the whole reach without devastating the population. Therefore, it was considered that the most meaningful comparative data could be obtained by determining the maximum standing crop at each reach. A visual subjective assessment for the area of highest standing crop was made. From this area, within the 10 m reach, 5 samples were taken, one of which represented the maximum standing crop for that reach. Where several macrophytic growths of algae were present, more than 5 samples were taken and where no obvious growth was present 5 areas of the substrata were sampled at random.

The method of harvesting employed, depended on the alga being sampled and on the substratum. Where the surface was loose, and the algae were not attached, a no. 13 cork borer (area of 380 mm^2) was pushed into the substratum thus isolating a column of water and plant material. Using a plastic tube attached to a large syringe, the contents of the column were removed and transferred to a clean McCartney bottle. Where moss protonema and algae were to be taken, the cork borer was driven approximately 10 mm into the substratum and the column of water drawn off and transferred to the container. The plug of moss protonema and algae could then be transferred separately. Filamentous species were harvested by pushing the borer into the substratum, thus cutting through the filaments and isolating a known area of algae. The material was transferred to the bottle as described above. The majority of reaches sampled had clay, silt, or flocculent precipitate as their substrate, and therefore, the techniques described above could be utilised. However, in some instances it was necessary to crop solid surfaces. Where possible, this was completed by removing the substratum, for example a stone, from the water, and the area to be sampled was marked out.

The material was then scraped off with a knife. When this was not possible the main flow of water was diverted from the sample area using a sheet of plastic and the sample either scraped or sucked off the surface.

From each area sampled, a small subsample was taken for microscopic examination. This was done so as to determine which species were present, and in what proportion the individual species were likely to contribute towards the total chlorophyll a extracted.

It was necessary to keep the samples cool during transport, especially over the summer period. If this was not done it was found that the flagellate species, in particular, were degraded before extraction could be carried out. The effectiveness of the technique for determining the maximum crop was tested at reach 1 where 50 samples were collected from a 10 m reach. The standing crop values ranged from 2.3 $\mu g\ mm^{2}$ to 0.1 $\mu g\ mm^{2}$ chlorophyll a, with a mean value of 1.9 $\pm$ S.D. 0.36 $\mu g\ mm^{2}$ chlorophyll a. In addition to these samples, 10 samples were taken which were visually considered to represent the maximum standing crop of algae for that reach. The mean value for these estimates was 2.1 $\pm$ S.D. 0.25 $\mu g\ mm^{2}$ chlorophyll a. These results indicate that the values recorded as the maximum standing crop were probably near enough to the true values to be sufficiently accurate for comparative purposes, both between different reaches and different samples.

2.62 Extraction of chlorophyll _a_ from field material

The extraction procedure was carried out immediately on return to the laboratory. Chlorophyll was extracted into 90% acetone and absorbance measured on a Perkin Elmer 402 recording spectrophotometer at 665 nm. As sediments invariably contain coloured degradation products of chlorophyll (Vallentyne, 1960) it is essential when making an estimation of the pigments of benthic communities, that either the crops must be separated from the sediment, (Eaton & Moss, 1966), or determinations must include a correction for the degradation products (Wetzel, 1964). It was probable that the reaches being sampled would contain considerable amounts of dead material, especially where moss protonema occurred. Therefore, as it was not practicable to separate the algae from the substrata, corrections were made to the final chlorophyll _a_ concentrations for the degradation products, using the method of Lorenzen (1967). The optical density of the extract was measured before and after acidification.

A problem specific to chlorophyll extractions on material from acid water, is that any residual acid on the sediment or algae, will cause immediate degradation of the chlorophyll to pheophytin on addition of the solvent. This was overcome by increasing the pH of the sample to 7.0 before the extraction took place. $Mg\ CO_3$ and Na OH were used for neutralization. $Mg\ CO_3$ was preferred because any excess NaOH caused a drift in absorbance at 665 and 750 nm.

The effectiveness of preventing degradation of the pigment by this method, was checked by determining the acid factor of a healthy culture of an acidophilic organisms growing at pH 2.7. The acid factor refers to the ratio of absorbance of the extract between 660 and 665 nm, before and after, acidification of the sample. The culture was divided into two, one aliquot was left at pH 2.7 and the second neutralized using $MgCO_3$. The samples were centrifuged, and the supernatant discarded. The chlorophyll a was extracted with 90% acetone and the absorbance of the extract recorded. The extract was then acidified with 1NHCl and the absorbance at 665 nm re-read. It was assumed that the culture contained little or no degradation products and therefore should give an acid factor in the region of 1.7, that being the figure given by Lorenzen (1967) and Marker (1972) for several other algal species. An acid factor of 1.66 indicated that the neutralization technique was successful. Where no treatment was used, the acid factor was 0.64, a figure within the range given by Marker which was assumed to represent phenophytin.

As chlorophyll determinations were to be carried out on several different forms of algal material, preliminary tests were made to establish both the most effective solvent and the most effective treatment of the sample prior to extraction. The combinations that were examined are given in Table 2.3. Plant material was sampled from three predetermined areas known to contain representative species, as given in sections A, B and C cf. Table 2.3. The samples

Table 2.3 Chlorophyll a determinations on field material.

solvent		treatment					
		no pre- treatment		ground with sand before extraction		sonification	
		1	2	1	2	1	2
90% acetone	A	0.56	0.02	0.57	0.002	0.56	0.02
	B	0.21	0.23	0.47	0.002	0.31	0.10
	C	0.18	0.21	0.42	0.002	0.31	0.11
90% methanol	A	0.53	0.01	0.54	0.02	0.54	0.01
	B	0.32	0.13	0.48	0.06	0.36	0.21
	C	0.20	0.22	0.39	0.04	0.33	0.07
90% hot methanol	A	0.42	0.12	0.53	0.001	0.21	0.17
	B	0.49	0.02	0.48	0.003	0.48	0.03
	C	0.34	0.10	0.40	0.01	0.33	0.12

(chorophyll results expressed as ug mm^{-2})

A = flagellated species and diatoms
B = filamentous species
C = moss protonema

1= first extraction
2= re- extraction

were then subdivided equally and the pre-extraction and extraction processes carried out. The material was re-extracted in order to determine the efficiency of the primary treatment. So as to avoid having too many different techniques in use at once, and to avoid the re-extraction procedure, it was decided that two combinations would provide adequate extraction for all categories A, B, C. Therefore 90% acetone, without pre-treatment, was used for samples containing species in category A and 90% acetone, with a pre-treatment of grinding, was used for any sample containing filamentous organisms.

As a result of the preliminary examination of neutralization and pre-treatment extraction, the following technique was implemented for all samples. The McCartney bottles containing the plant material and the sediment were filled with distilled water and after pH adjustments to 7.0, cencrifuged at 4000 r.p.m. for 5 minutes. The supernatant was discarded and where grinding was necessary, the material transferred to a pestle and mortar, and then ground with sand. This material was then returned to the sample bottle and rewashed to ensure that no acid remained. 10 ml of 90% v/v aqueous analar acetone were added to the samples and the bottles sealed and stored in the dark for 24 hours, at 4^{o}C. A period of 24 hours was necessary in order to allow time for complete extraction to take place. On completion of the extraction process, the sealed bottles were centrifuged at 4000 r.p.m. for 5 minutes and the clear extract decanted off into a volumetric flask and made up to volume.

With the exception of the grinding process all other steps were carried out in the same McCartney bottle, in order to reduce loss of sample during transfer from one vessel to another. Using aliquotes of the extract in 1 cm cuvettes, the absorbance was read at 665 and 750 nm, on a Perkin-Elmer recording spectrophotometer. The extract was then acidified directly in the spectrophtometer cell, with 2 drops of 1N HCl and mixed for several seconds before re-reading at the same wavelengths. Where necessary, the sample was diluted 10 times before the initial reading was taken. The optical density was measured at 750 nm, as an approximate measurement of the background absorption by material other than chlorophyll a. This figure was subtracted from the 665 nm reading, which was used for the calculation of chlorophyll a concentration.

The measurements obtained at 665 nm before and after acidification were substituted in the general equation of Lorenzen (1967).

$$\text{Chlorophyll } \underline{a} = (D_b - D_a) \times \left[R/_R - 1 \right] \times v/_1 \times 10^3/_{ac}$$

Where Chlorophyll a = concentration of chlorophyll a in µg/sample

D_a = optical density of the extract after acidification

D_b = optical density of extract before acidification

ac = specific absorption coefficient for chlorophyll a

v = volume of solvent used in extraction (ml)

l = path length of the spectrophotometer cell in cm

R = D_b/D_a for pure chlprophyll a.

using 90% acetone the values substituted in the equation were:

$$ac = 84,\ v = 10\ \text{ml},\ 1 = 1\ \text{cm and } R = 2.43.$$

The specific absorption coefficient of Talling & Driver (1963) was adopted because of the possible presence of chlorophyll b and c. This gives an empirical coefficient of 11.9 for 90% acetone. Thus the final equation used was:

$$\text{Chl } \underline{a}\ \mu\text{g unit area}^{-1} = 11.9 \frac{\left[2.43\ D_a - D_b\right]}{\text{area sampled}}\ 10\ R\ .$$

For tests carried out using 90% methanol, the empirical coefficient of 13.9 was used.

The chlorophyll content of each of the five samples taken from each reach was thus determined and the largest chlorophyll concentration per unit area was recorded as the maximum standing crop for that reach.

2.7 Culture techniques

2.71 Equipment and environmental conditions

'Pyrex' glassware was used for all culture and experimental work. This was soaked in 10% HCl for 24 hours and rinsed six times in distilled water to remove any absorbed trace elements. The non graduated glassware was dried at 108°C for at least 2 hours before use. This served both to dry and partially sterilize the equipment. All chemicals and solvents used were of analar grade, so as to reduce contamination of ions as much as possible.

The majority of cultures were grown in 30 ml of medium, in 100 ml flasks. When large amounts of inoculum were needed, 100 ml of medium in 250 ml flasks were used. Except where specified, all cultures were grown at $15^{o}C + 1^{o}C$, under constant fluorescent light of approximately 2000 lux. Stock cultures were maintained either in standing culture, or in water cooled shake tanks. The gentle shaking action of these tanks promoted the growth of most species, and therefore was used mainly for experimental work.

2.72 Isolation techniques and preparation of media

An effort was made to collect and culture as many of the acidiophilic species as possible. With the exception of one or two species this proved to be difficult and in some cases impossible. The cultures were used as an aid to identification, and also as a source of test organisms for experimental work. The six species maintained successfully for a long period of time in unialgal culture, were all isolated from Brandon Pithouse Acid Stream.

As the organisms were isolated from a fairly low nutrient environment, it was felt that a standard, low nutrient, mineral medium should be used for culturing. Several high and low nutrient media were tried, of these a modification of Chu (1942) 10 liquid medium was found to be satisfactory for the growth of most organisms (see Table 2.4).

Analysis of the acidic water during the general surveys indicated that nitrogen was present mainly as NH_4-N, rather than NO_3-N (see 4.1), therefore, in addition to the NO_3 already in the medium NH_4SO_4 was added. This improved the growth of several species, in particular, Gloeochrysis turfosa, Chlamydomonas spp. and Euglena mutabilis.

Table 2.4 Composition of medium developed for growth of acid stream species (all concentrations in mg l^{-1}).

Major salts	KH_2PO_4	7.8
	$MgSO_4.7H_2O$	25.0
	$Ca(NO_3)_2$	40.0
	$NaHCO_3$	15.8
	Na_2SiO_3	10.9
	$(NH_4)_2SO_4$	76.0
	Fe (added from Fe.EDTA stock)	2.0

Microelements 'C' stock (1 ml l^{-1}) of Kratz & Myers (1955)

Batches of media (21) were made up using double distilled water and the pH corrected with 1N and 0.1 NH_2SO_4 and NaOH. The pH of the medium remained stable over the range pH 1.5 to 4.0, but above this value it tended to drift towards neutral unless corrected regularly. In order to avoid the use of chemicals not likely to be present in the natural water, other buffering systems were not considered. Attempts to obtain axenic cultures were unsuccessful, although the number of bacterial and fungal contaminants remained minimal as long as regular subculturing was employed.

The isolation of species was made easier by the natural colonization of the organism in the stream. It was not uncommon to find areas of stream completely dominated by one species, almost forming a unialgal population. Where this occurred, samples were transferred directly into medium and left to grow. If a mixed population was present, efforts were made to isolate species, either by micropipetting, serial dilution, or streaking onto agar plates made up with acidified medium. Additonal samples of the mixed population were put into media, and left in the hope that one species would grow and dominate the rest.

Species that produced a motile stage were put into flasks in the dark and left overnight. This invariably stimulated the production of the motile stage which could then be pipetted from the surface. As the species varied in their tolerance to pH, inoculation of medium over a pH range proved to be a successful method of isolation, especially for the more acidophilic species, eg. Euglena mutabilis.

Agar was used for the growth and isolation of some species, in addition to liquid medium. The medium was prepared by the addition of agar to boiling acidified Chu 10. After the agar had dissolved it was poured into plastic petri dishes and stored at $4^{o}C$. Boiling of the media for 15 minutes was used in preference to autoclaving, because it was found that autoclaving acidified agar and prevented it from setting.

2.8 Growth response experiments

2.81 Introduction

Growth of an organism was determined by chlorophyll a extraction methods at the end of the experimental period and expressed as $\mu g\ l^{-1}$ chlorophyll a. Microscopic examinations were also carried out to establish whether any morphological changes had occurred. An attempt was made to maintain simple, uniform, experimental conditions, which could be related as nearly as possible to the field conditions. This was achieved by the used of a basic defined mineral medium, buffered with acids and alkaline solutions which occurred naturally in the environment. Four replicates of each treatment were prepared and most experiments were repeated at least once.

2.82 Experimental equipment, media and conditions

All experiments were conducted in 100 ml pyrex conical flasks, previously treated as in 2.71. All other glassware was similarly treated before use, in order to reduce contamination by non desirable trace elements. The medium that was used in all experiments was based on the Chu 10, as given in section 2.72. For experiments where pH tolerance was being investigated, the basic medium was used. Investigations concerning the reaction of organisms to varying concentrations of an element, required a slight adjustment of other elements in addition to the one being examined. Where a single element was concerned, the chloride or sulphate salt was used. Controls using NaCl and Na_2SO_4 were carried out and these salts were found to have no effect on growth at the concentrations used.

Acid stream water was passed through membrane filters before addition to the medium. In all experiments 30 ml of medium were added to the flasks and allowed to equilibriate at 15°C. Replicas of each treatment were then inoculated, cotton wool bungs were placed in the necks of the flasks, and the flasks were then placed in the shake tanks at 15°C and 2000 lux.

2.83 Size of inoculum

The material to be used as the inoculum for the experiments was acclimatized to the culture conditions for at least 24 hours. As both fresh field material and stock cultures were used, tests were carried out with *Hormidium rivulare* to ascertain whether there was any difference in growth response between field and culture material. The results of the preliminary tests are given in 6.24 and 6.33.

The size of inoculum was also tested using the most pH sensitive organism, *H. rivulare*, the results of these experiments are also given in 6.24. As this species is both filamentous and mucilaginous, all attempts to produce a homogenous suspension of filaments were unsuccessful and therefore, small amounts of filaments were separated manually. A 'normal' inoculum size was adopted for the experiments as a result of the tests carried out in 6.24. As all the other species used in experiments were singled celled, a suspension of these could be accurately pipetted into the experimental flasks. An inoculum size of equivalent to approximately 40 $\mu g\ l^{-1}$ chlorophyll a, was used for all five species.

2.84 pH tolerance experiments

The general methods described in section 2.81 were employed. Several species were tested over a pH range from 1.5 to 7.0 and the amount of growth was determined by chlorophyll extraction at the end of the experiment.

The incubation time of each experiment depended on the organism being tested. For Euglena mutabilis a period of 10 days was employed, as this organism had a slow growth rate in culture. The other species used included Hormidium spp., Gloeochrysis turfosa and Chlamydomonas applanata var. acidophila and these were usually left for 7 days before being harvested (6.23)

The organisms were exposed to the following pH values: 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0. Where preliminary tests were carried out several pH values could be omitted eg. Hormidium rivulare did not grow below pH 2.5. The individual values were adjusted to the required pH by a dropwise addition of 1N and 0.1N H_2SO_4 to the medium.

The liquid was continuously stirred with a magnetic stirrer and the pH was measured on an E.I.L. pH meter. Where necessary 1N and 0.1 NaOH were added in a similar manner. The pH values were adjusted to + 0.02 of a unit. The medium was inoculated and left in the culture tanks as described in 2.81 for the required time.

As there was a tendency for the pH to drift, each flask was checked on alternate days and when necessary readjusted to the correct value. For values of 1.3 to 3.5 the pH was maintained at ± 0.05 of a unit, and for values between 4.0 and 7.0 ± 0.2 of a unit. Care was taken to rinse the electrode with distilled water between measurements, so as to avoid carry over of material from one flask to the next.

2.85 Heavy metal tolerance

The methods and conditions described in section 2.82 were adopted for investigations into the effects of Zn and Cu on acidophilic algae. Two test organisms were subjected to varying amounts of Zn (as $ZnSO_4$) and Cu (as $CuSO_4$) at a range of pH values. Quantitative estimations of growth were determined by chlorophyll extraction techniques (see section 2.86), after exposure to the metals for 7 days. Adjustments to pH were made as described in section 2.83 and the final concentrations of the metal under investigation were made up at each pH value of the range. The concentrations which were tested, depended on both the test organisms and the element being examined. The levels were initially decided by reference to the field data, consequently in order to accommodate field values, the difference between concentrations within the series was often large. Also, the number of levels in a series was at times determined by the amount of space available in the culture tanks.

All dilutions were carried out in volumetric flasks 5 g l^{-1} stocks of the elements were used in order to obtain the final concentration required without diluting the medium by more than 2 percent. An effort was made to determine the effect of varying the concentrations of several other metals, besides the heavy metals, and to see what effect they had on growth at low pH and high heavy metal concentrations (eg. Ca and Mg). The same procedures as described for the heavy metal tolerance experiments were used in these experiments.

2.86 Harvesting and estimation of growth

At the end of an experiment the algae were harvested by vacuum filtration through 25 mm diameter glass fibre papers. The efficiency of these filters was tested by re-filtering the supernatant through membrane filters. No material was found to pass through the glassfibre.

The algal material on the paper was then placed in a McCartney bottle and the amount of growth estimated by pigment extraction. A known volume of solvent (90% acetone or methanol) was added to the bottles so as to cover the algal material. Usually 5 or 10 ml of solvent was added depending on the amount of material present. 90% acetone was used for the total extraction of pigment from the following organisms: *Euglena mutabilis*, *Chlamydomonas applanata* var. *acidophila* and Gloeochrysis turfosa. The last organism was surrounded by mucilage and pretreatment by grinding with sand had to be applied to complete extraction. Complete extraction of these species occurred usually within one hour, although it was often convenient to leave them overnight at 4°C in the dark.

Hormidium spp. and Stichococcus bacillaris were extracted in hot methanol. After the addition of the methanol the tops of the bottles were sealed to avoid evaporation of the solvent during heating. These were then placed in a water bath at approximately 70°C for a few minutes until the pigment had been extracted (usually 5 minutes was sufficient). The bottles were then removed and allowed to cool at 4°C in the dark. This modification of the hot methanol technique was preferred to boiling the samples individually, as suggested in the I.B.P. Handbook since many extractions could be carried out together, depending on the size of the water bath. The technique was carefully checked against the recommended method before use, but there was no evidence to suggest that there was any loss of chlorophyll a due to degradation, or that incomplete extraction was taking place.

Following extraction all samples were passed through glassfibre filters or centrifuged to remove any debris and then made up to volume. The absorbance of the samples was read at 665 nm and 750 nm on a Perkin Elmer 402 recording spectrophotometer, using 100 and 400 mm cells, (see section 2.62). The amount of chlorophyll a per sample was determined by applying the following equation:

$$\text{Chl } \underline{a} \text{ } \mu g \text{ per sample} = \text{O.D.} \times 11.9 \times \frac{v}{l} \quad \text{(for acetone, 90\%)}$$

$$= \text{O.D.} \times 13.9 \times v/_{l} \quad \text{(for methanol, 90\%)}$$

Where O.D. = absorbance at 665 nm

v = volume of solvent

l = path length of the spectrophotometer cells

As only a measurement of the live material was required no allowance was made for chlorophyll degradation products.

Statistical analysis and computing

All statistical methods were based on those suggested by Bailey (1959) and Siegel (1956). Apart from the determination of means, standard deviations and standard error all analyses were carried out using an IBM 360/67 and 370/168 computer (NUMAC). For the most part the SPSS (Nie *et al*., 1975) packaged programmes were made where necessary.

3. DISTRIBUTION AND DESCRIPTION OF SITES AND STREAMS IN ENGLAND

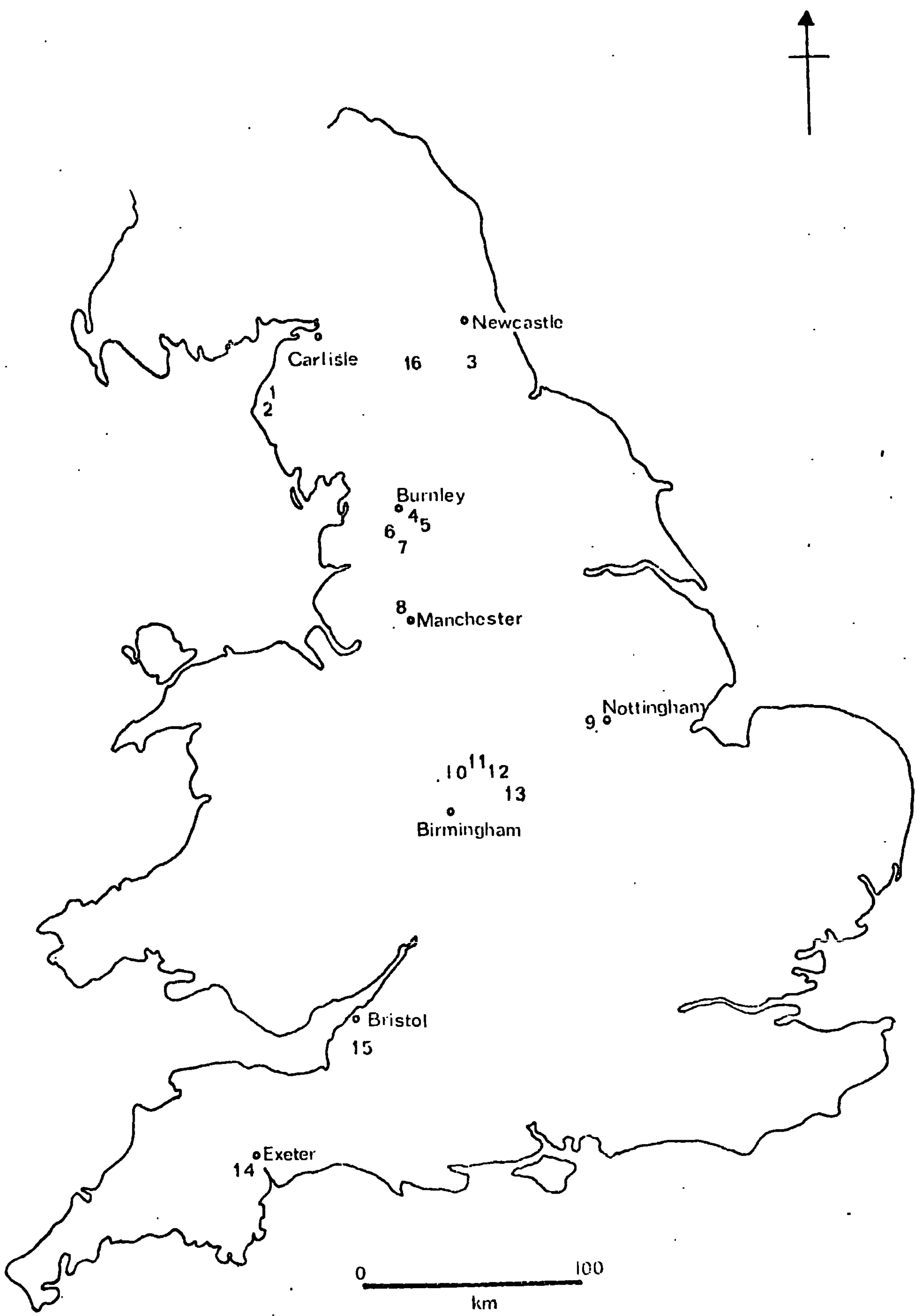
3.1 Distribution of sites

The distribution of the sites surveyed, with water at and below pH 3.0, are given in Fig. 3.1. The exact location of the sites together with the topography and geological description of the stream complexes, are given in Tables 3.1 and 3.2. Fourteen of the sites visited were associated with coalfields, one was a disused barytes mine, (site 14), and one was an intermittent Industrial source (site 15). As previously mentioned, although Dowgang (site 16) is associated with a coal seam, it was found to run at pH 3.0 only at times of low flow.

The geography of the sites, including the approximate dimensions of the individual stream complexes and pools, are given in Table 3.1. The number of separate stream complexes, sampling reaches and pools are given for each site. The length of the streams varied from a few metres to about 1000 m and their widths from 20 to 1500 mm (site 7 and 6 respectively). Depth of water within a stream varied greatly from approximately 5 to 800 mm, invariably the deeper sections were very slow flowing.

The approximate dimensions of the major pools given in Table 3.1, varied considerably from small, shallow pools (site no. 1), to large deep ponds (sites 11 and 6). The depth of the larger ponds was not estimated because collection of

Fig.3.1 Location of acid mine drainage sites in England with water ≤ pH 3.0



site No.	name	grid reference	no. stream complexes	approx. stream dimensions: total length including (m) pools	approx. stream dimensions: breadth (mm)	approx. stream dimensions: depth (mm)	no. major pools	approx. dimensions of largest pool within stream complex: length (m)	approx. dimensions of largest pool within stream complex: breadth (m)	no. of side flushes per stream complex	total no. reaches per complex (A+B, stream + pool)	total no. reaches per site
1	Walkmill	NY 007188	1	30	200-500	5-60	1	6	5	2	3	3
2	Oatlands	NY 026216	1	40	100-300	5-50	1	12	6	0	2	2
3	Brandon Pithouse	NZ 213404	2	385 25	100-400 150-1000	5-60 5-400	1 0	15	12	0 1	3 1	4
4	Rowley	SD 867333	2	200 175	150-500 200-700	5-60 5-50	0 0			2 2	4 3	7
5	Deerplay	SD 869266	1	250	250-1000	5-150	0			1	3	3
6	Chisnall Hall	SD 553128	3	1000 20 25	500-1500 100-300 200-800	5-800 5-30 10-150	2 1 0	200 10	50 5	4 0 1	7 1 1	9
7	Welsh Whittle	SD 545135	1	5	20-50	5-15	0			1	1	1
8	Gibfield	SD 661023	1	100	100-800	5-50	1	12	8	3	4	4
9	Denby	SK 391483	2	100 70	100-600 100-500	5-60 5-30	0 0			2 1	4 2	6
10	Cannock open-cast	SJ 990083	1	15	100-400	5-30	1	7	5	0	1	1
11	Polesworth	SK 257038	1	400	500-1000	5-100	1	300	80	0	3	3
12	Kingsbury	SP 233986	1	250	100-500	5-40	0			1	3	3
13	Birch Coppice	SK 255001	1	110	100-200	5-50	2	15	12	1	3	3
14	Bridford	SX 816854	2	40 40	100-1000 50-300	5-150 10-200	0 1	25	15	0 0	2 1	3
15	Industrial	Nr. Mine-Head	1	2000	1000-2000	500-1000	0			0	3	3
16	Dowgang burn	NY 777428	1	300 15	200-1500 100-500	10-150 5-50	0 0			0 0	2 1	3

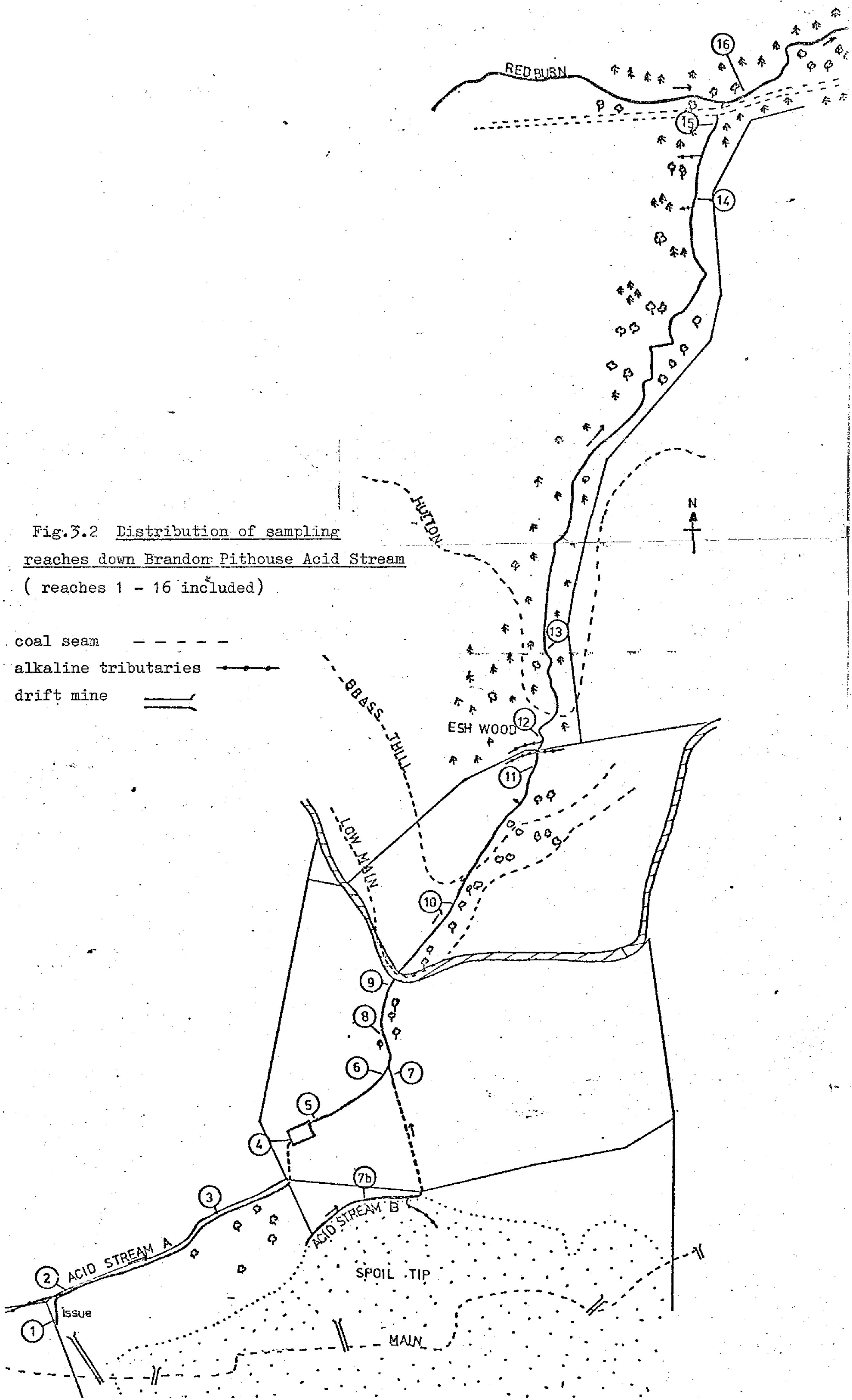


Fig.3.2 Distribution of sampling reaches down Brandon Pithouse Acid Stream (reaches 1 - 16 included)

site no.	name	no. stream complexes	main source	predominant stream + pool substratum	predominant grades of substratum (Wentworth scale)	presence of Iron oxide precipitate
1	Walkmill	1	Sp, Se	clay, shale, sandstone	silt, sm. pebbles	C
2	Oatlands	1	Se	shale, sandstone clay	silt, sand	
3	Brandon Pithouse	2	Sp	clay, shale, sandstone	clay, sm. pebbles	
			Se		clay, sand	
4	Rowley	2	Se	shale, clay, sandstone	silt, sand, sm. pebbles	F, C
			Se		silt, sand, sm. & med. pebbles	F
5	Deerplay	1	Sp	shale, clay	silt, sand, sm. & med. pebbles sm. & lg. boulders	C
6	Chisnall Hall	3	Se	clay, shale, sandstone	clay, silt	F
			Se		clay, silt	
			Se		silt, clay	F
7	Welsh Whittle	1	Se	clay	clay, silt	
8	Gibfield	1	Se	clay, shale	clay, silt	F
9	Denby	2	Se	clay	clay, silt	F
			Se	shale, clay, sandstone	silt, sand, sm. pebbles	F
10	Cannock Opencast	1	Se	clay	silt, clay	
11	Polesworth	1	Se	shale, clay, sandstone	silt, clay	
12	Kingsbury	1	Se	clay, red sandstone	clay, silt	F, C
13	Birch Coppice	1	Se	clay, shale, sandstone	silt, clay	
14	Bridford	2	Sp	red sandstone, shale	silt, sm. pebbles	C
			Sp		silt, sand	
15	Nr. Minehead	1	Industrial	clay	silt	
16	Dowgang	1	Se, Sp	sandstone, shale	silt, pebbles, sm. & med. boulders	C, F

samples was restricted to the shallow water at the edge of the ponds. At several sites (sites 2, 3, 8, 13 and 14) smaller man-made holding lagoons had been excavated in an effort to control the spread of the acid drainage water. The larger ponds at sites 11 and 6 had completely different appearances. As mentioned in 7.28, the water at site 11 was clear whereas the pond water at site 6 was bright red in colour due to the very large concentrations of ferric compounds present in the water.

3.2 Geology and history of sites

3.21 Source of acid water from coal mine

Coal measures of the upper and low carboniferous period are usually associated with a series of clays, shales, grits, sandstones and ironstones. Limestones are with a few exceptions, noticeably absent from the coal seams. Sulphur commonly occurs in varying amounts in most coal seams, usually in the form of iron pyrites. Other mineral ores in particular aluminium silicate, are also found in the veins immediately around the coal seams. During the process of coal mining, these minerals, unless present in large amounts, are treated as waste along with the other non carboniferous material such as the shales and clays, and are either left underground, or are brought to the surface and dumped. Many of the streams surveyed were situated at the base of the waste tips and often the bed of the stream consisted of a mixture of different substrata from the various depths of the mine.

The main source of most of the streams was from seepage from the spoil heaps (Table 3.2), but six originated in part from springs in the immediate area of the tip. In all but one of the latter (site 1), the water had been diverted through a pipe. In these instances it was difficult to determine with any certainty whether the source was a nature spring, or whether the pipe had been laid to drain the old mine shafts.

It is possible that several of the seepages originated from underground springs, rather than water percolating through the spoil heap. If this was the case, then it is likely that the point of issue of the water had been diverted by the spoil material, so that its exit from the tip was in the form of a slow seepage rather than the continuous flow from which it originated. Where seepages occurred, they were usually represented by several small issues of acid water combining to form a larger stream. These were invariably led away from the tip by man-made ditches and in the case of sites 4 and 12, the water was then neutralized in treatment lagoons.

3.22 Substrata

The principal components of the substrata of the streams are given in Table 3.2. The substrata were very similar, consisting mainly of clay and shale, with sandstone occurring less frequently. The particle size of the substrata, as determined by the Wentworth scale, (Stranhler, 1971), varied from clay to boulders. However a mixture of clay, silt and sand was typical. The most variable substrata size was found at sites 4 and 16, where it ranged from silt to large boulders which often formed waterfalls. The substrata at many sites were often mixed with friable and compacted precipitate, usually of ferric compounds. Although the majority of iron in solution does not precipitate out below pH 3.0 some ferric iron when present in very large amounts will gradually precipitate out of solution, thus partly covering the stream bed. Because of the nature of the substrata and the presence of friable precipitates, the stream beds were rather unstable and subject to considerable erosion under high flow. As many of the streams were located close to the spoil heaps, run-off from the tips into the streams at times of heavy rainfall, could have quite devastating effects on the stability of the bed. Of the sites surveyed, only sites 6, 12 and 14 would be relatively free from the extreme scouring effects of high flow.

3.23 History

It was almost impossible to determine how long these acid streams had been in existence, as it appears that no specific records have been kept for any of them. Knowledge of the age of the mine may be of some advantage, but in many cases information is rather scattered and often dates are vague. Several mines are likely to have been in operation for over 100 years (sites, 3, 4, 6, 9, and 11) but whether there has been acid running for that length of time is unknown. With the exception of sites 10 and 13, all other sites have been closed down for a number of years, and sites 5 and 8 have been reclaimed and grassed. It is likely that the springs which formed several of the streams were in existence before the mining activities began (eg. sites 1 and 3). However, this does not mean that the spring water had a low pH.

3.24 Sites not associated with coal mining

Of the two sites not associated with coal fields, the barytes mine (site 14) was situated on a strata of predominantly red sandstone and shale, with the barytes vein occurring in a natural fault. The source of the acid most likely comes from the oxidation of the barytes and to some extent iron pyrites associated with the vein. The main stream originated from a spring at the base of a rock face, presumably this spring had been in existence before the mining. The second source was from drainage from within the mine and flowed into a lagoon. The shafts had been abondoned for several years after the minerals became uneconomical to mine. As the mine was privately owned it was not possible to find out when mining had commenced, although the size of the mining area suggested that it had been operational for at least 50 years.

The industrial effluent originated from a munition factory and consisted of sulphuric and nitric acid. The effluent was released into a draining ditch which eventually flows into the sea. The factory would not allow the exact position of the stream to be given, nor would it give information concerning the frequency of discharge. However, the large changes in flora (see 4.62) suggested that the releases were infrequent rather than on a regular basis.

3.3 Geology and history of Brandon Pithouse Acid Stream

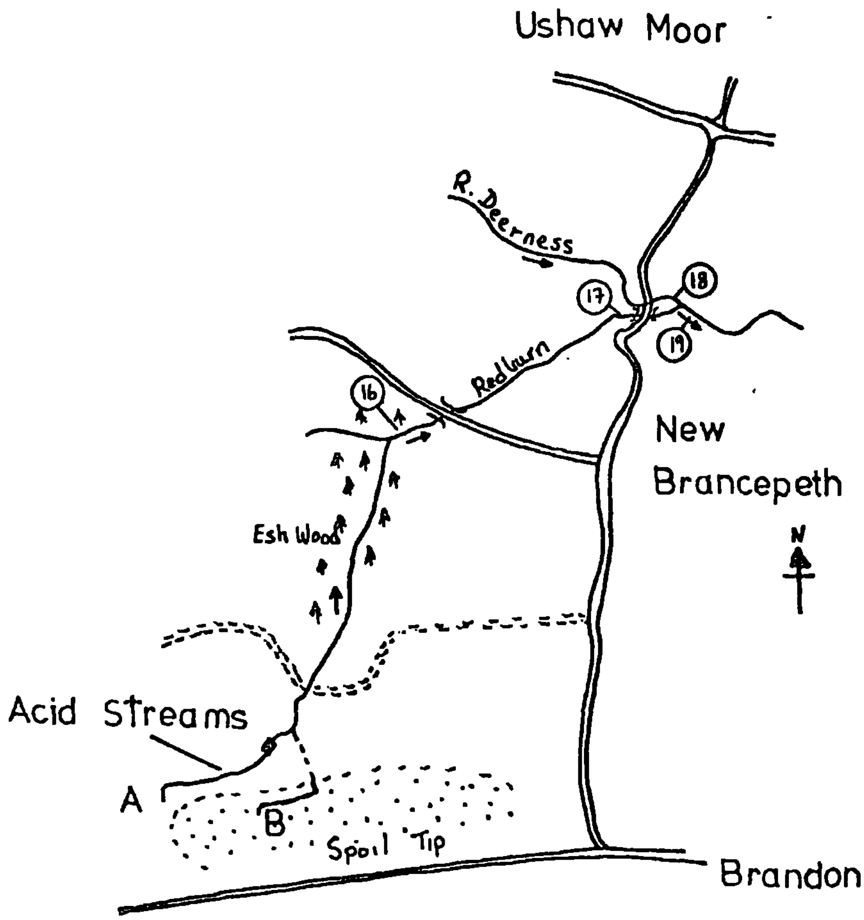
3.31 Location

As mentioned in 1.9, of the 16 sites surveyed, one site at Brandon Pithouse Colliery (site 3), was studied in detail. The colliery is situated on a north west facing hillside, at an altitude of 800 ft, approximately 5 miles from Durham City and in the Urban District of Brandon and Byshottles. The acid water draining the colliery, flows from the tip down a valley, where it is mixed with other non acidic streams, thus creating a pH gradient of 2.6 to 8.0. Except in discussion of the general surveys of streams, the stream draining Pithouse Colliery will be referred to in the text as Brandon Pithouse Acid Stream and not site 3 as in Table 3.1.

3.32 Geology

Brandon Pithouse is situated on the western extreme of the Durham Coalfield, which was deposited during the Upper Carboniferous period. The rock types associated with the coal seams and which are therefore present in the spoil heaps, consist mainly of fine ground sandstones, shales, and fire clays. Iron pyrites and aluminium silicate are commonly associated with the coal seams together with other mineral ores such as sphalerite, barytes and witherite (Woodward, 1876). During the sinking of the shafts to the deeper seams, such as the Brass Thill and Tilley, and drifts into the more easily accessible outcrops of the Five and Main Quater, (see Fig. 3.2) layers of non carboniferous material were disturbed and brought to the surface as waste. The

Fig. 3.2 Map of Brandon Pithouse Acid Stream, from source to confluence with River Deerness.(reaches 16-19 included)



exposure of these mineral ores to air and water, underground and on the tips, gives the water its chemical characteristics. The various seams which have been exploited at Brandon all have very similar surrounding strata. The measures of the middle coal seams are surrounded by a predominance of shale, siltstone and subordinate sandstone. The Tilley, which was the deepest seam mined, is situated near the lower coal measures and is surrounded by a predominance of sandstone, shale, and grit. The underlying strata below the lower measures, consist of Durham Millstone Grit and rocks of the Upper Limestone group, however, it is doubtful whether these rocks will have been exposed by mining activity. Another possible source of mineral ores is from a natural fault which transects the coal seams at several points along the hillside.

The occurrence of witherite ($BaCO_3$) in the immediate vicinity of the mine is noteworthy. This mineral is considered reasonably rare and several decades ago was mined only a few miles away at Brancepeth (Wilson, et al. 1922). Analysis of the mine water did not show significant amounts of Barium. The possible reason for this could be that the sulphate salt of Barium is insoluble at low pH and may be precipitating out underground and therefore would not be detected at the surface.

3.33 Historical background and source of acid water

Records suggest that coal mining in the area has been carried out since the 13th century, when monks worked the more easily accessible outcrops in the Durham area, (Dewdney, 1970). Specific reference was made to mining of Pithouse colliery in 1856, but Diocesan records of 1838 show the existence of shafts to the west and east of the present day pit. Surveys of the area indicate that a considerable amount of mining had occurred prior to the sinking of the main shafts in the 1900s. It is likely that some of the mines which occur where the coal seams are near the surface, have been in existence since the early 1800's, (I. Green, 1975 pers. comm.). There is evidence of drifts into several of the major seams which outcrop along the hillside. The Five Quater, Main, Brass Thill and Low Main were all mined during this period. The presence of many of these drift mines was not shown on the O.S. maps drawn up in 1897, probably because as soon as the drift was finished it was filled in and would not therefore be externally obvious. It was not until after the geological maps were drawn up that many of these drifts were recorded.

After drift mining techniques had diminished the coal supplies in the more easily accessible seams, the main shaft was sunk down to the Tilley seam in about 1926. It was the practice at the time to sink the main shaft to the lowest seam and then work the seams in ascending order. Consequently, since 1926, many of the measures represented in Fig. 3.2 have been worked. The colliery was finally closed in 1966, leaving a spoil heap of approximately 800 metres across. In 1970 contractors began to reclaim the coal still present in the spoil. This work is still in progress and eventually the complete tip is to be landscaped and probably returned to agriculture. During the reclamation it is likely that the spoil will be covered by clay and top soil before being planted. It will be interesting to see if this reduces the amount of acid produced, as this technique is recommended as a method of abatement of acid water.

The cause of the acid water is undoubtedly due to the oxidation of pyrites and consequently the production of sulphuric acid (see 1.22). The pyrites is either directly associated with the coal or in the adjacent veins lying around the marine bands. The seam at Brandon Pithouse consists of high grade coal of low sulphur content, so it is likely that the pyrites is in the adjacent rocks (J. C. Trickett, 1973, pers. comm.).

The origin of the acidic drainage water is uncertain because the whole area which supported many drifts, has been covered by the present spoil heap. One possible source is that rain water percolates through the spoil and is exposed to iron pyrites and other waste minerals, thus leading to the production of acid water. However, if the water is passing through the spoil, it should contain its own water table. Borings made by Taylor & Attewell (1968) did not locate any such water table. Further indirect evidence which disproves this possibility is that if the source of acid was due to percolation, then it would be likely that acid seepage would occur at several points along the tip.

However, this is not found to be so, because although there are several intermittent issues from the tip these are not acidic.

The other possible source is drainage from the old drift mines. Stream B is likely to originate from a drift in the Main seam which is now covered by the spoil heap. Maps of the site in 1897 showed a natural spring in the area in which there was a drift. This spring could be the source of water which comes in contact with pyritic material exposed when the drift was excavated.

The source of stream A is also situated directly below an old drift into the Five Quater and Main seams. This stream issues at a fairly constant rate from a clay pipe (see Table 5.1). The presence of the pipe suggests that water was draining from a drift before it was covered over. It was common practice to pipe away the drainage water from drifts before they were closed, in order to avoid a build up in the water table which could flood other working mines. Like stream B, it is possible that a natural spring was disturbed during the excavations, and it continued to flow through the exposed minerals after the mine was closed. The volume of water flowing from the pipe is so constant (see 5.2) that it strengthens the suggestion that the source is from a deep spring. Only after several months of above average rainfall does the discharge alter significantly. The presence of a natural fault in the coal seam above stream A could explain the relatively high concentrations of heavy metals present in the acid water.

There are no records of when the acid water first occurred. Although the position of the source of stream A was different, its approximate present course has been shown on maps since 1838. There is no evidence to suggest that it was running acid at the time, or that the drifts from which it is presumed to have originated had definitely been made in the positions shown on the later maps. However, as explained earlier in this section, the drifts could have been filled in by 1838 and therefore it is possible that the stream may have been acid for about 150 years; it is almost certain that it has been acid for 50 years (I. Green, pers. comm.).

3.4 Topography of Brandon Pithouse Acid Streams

The topographical details of each reach are summarized in Table 3.3. The two streams at Pithouse colliery issue from the base of a large waste heap and flow down the valley for 3.3 km in a northerly direction to Redburn and eventually the River Deerness at Ushaw Moor, (see Fig. 3.2).

3.41 Stream A, reaches 1-6.

Acid stream A flows from an earthenware pipe at a constant pH of 2.6 and except after a period of heavy rainfall for several weeks, the total discharge remains constant (see 5.2). The width and depth of the stream remains fairly constant throughout its length, varying from 150 to 600 mm in width, and 5 to 150 mm in depth. The stream is generally fast flowing, with the occurrence of a few small

reach no.	computor no.	stream width (m)	stream depth (mm)	predominant substratum type	grade of substratum (Wentworth scale)	gradient (mm)	Fe ppt. (1-3)	shading (1-5)
1	0127.01	0.5	5-60	clay	clay, silt	500	-	1
2	0127.02	0.3	5-40	clay	clay	400	-	2
3	0127.03	0.4	10-40	shale, clay	sm. pebbles,clay	100	-	4
4	0127.04	1.2	10-150	shale, clay,sandstone	sm. pebbles,silt	300	-	1
5	0127.05	2.0	10-50	shale,clay	lg. boulder,clay	2000	-	1
6	0127.06	0.8	5-100	shale, clay	pebbles,silt,sand	200	-	1
7	0127.07	0.7	5-50	clay	clay,silt	450	3	3
7b		1.7	10-800	shale, clay, coal	sand,sm.pebbles, silt	10	-	1
8	0127.08	0.6	5-150	sandstone,clay,shale	lg.boulder,silt, clay	1000	3	4
9	0127.09	0.8	10-60	sandstone,clay,shale	sm.boulder,clay	200	2	2
10	0127.10	0.4	5-100	sandstone,clay	silt,sand	300	-	1
11	0127.11	0.5	20-75	sandstone,clay,shale	sand, silt	80	-	1
12	0127.12	0.7	40-100	clay, sandstone	silt,clay	90	3	1
13	0127.13	0.8	10-90	clay, sandstone shale	silt,clay	300	3	1
14	0127.14	1.0	20-80	shale, sandstone	sm.pebbles,cobbles	300	3	2
15	0127.15	1.2	100-200	clay, shale,sandstone	silt,sand	250	3	3
16	0022.03	0.9	20-200	shale, clay	sm.cobbles,pebbles,	120	1/2	2
17	0022.06	2.0	20-150	shale, sandstone	cobbles,pebbles	150	-	2
18	0005.05	6.0	100-300	shale, sandstone	cobbles, pebbles, sm.boulders	100	-	1
19	0005.06	6.0	70-300	shale, sandstone	cobbles, sm.boulder, pebbles	120	-	1

pools and a section where the water flows through a bed of moss.

For a stretch of approximately 40 m below the source, the stream receives a considerable amount by run-off from the tip during times of heavy rainfall. The floods also cause a considerable amount of erosion lower down the stream, although the effect is not quite as dramatic as that observed at reach 2.

The stream then flows though a small coppice where the substrata change from clay to medium and small sized pebbles, and in summer the algae are subjected to a considerable amount of shading. The water is then piped under part of a field for 20 m into a small holding reservoir. The reservoir was built early this century but for what purpose is not known. *Drepanocladus fluitans* covered the bottom of the reservoir throughout the study period. This moss often becomes covered with the silt washed in during floods. The reservoir acts as a buffer for the rest of the stream, both by controlling the flow during floods and also retaining the water long enough to allow silt to settle out.

The stream continues for a further 50 m, through arable land, before it is joined by acid stream B. Stream A has run continuously as far as the reservoir for the three years of study and with the exception of the reservoir the water has never frozen.

3.42 Stream B, reaches 7 and 7b

Stream B is formed from several acid seepages at the side and toe of the tip. These collect in a ditch and form a slow flowing stream, part of which at times could be classed as stagnant. The stream is joined by alkaline surface water after 20 m. Where the pH rises above 3.5 hydrated iron (Fe+++) and aluminium oxides are precipitated out, colouring the stream bed yellow-orange. The water then passes through a pipe to join stream A at reach 7. The volume of water from the two streams is, under normal circumstances, approximately the same, although there is much greater variation from stream B because of surface run-off.

3.43 Downstream of confluence of stream A and B (reaches 8-12)

At the confluence of the two streams (reach 8), the pH increases and large amounts of hydrated iron oxide precipitate out, changing the characteristics of the stream bed for about 30 m from clay to a more unstable substrate of friable and compacted ferric hydroxide precipitate.. For 30 m below the confluence and at intervals from there until it reaches Eshwood approximately 200 m below the confluence there is some shading by large teees. This section of the stream runs through agricultural land and is subjected to intermittent nutrient enrichment by run-off from the fields.

During a period of low flow a small acid seepage was observed at reach 10. The source of this acid is not known but was assumed to emanate from an outcrop of the Brass Thill seam (so called because of the large amounts of pyritic material present in the seam). The pH and chemistry of the seepage could not be determined because the effluent ran directly into the stream from its underground source.

From reach 10 the water course becomes diverse as it flows through a bog of Drepanocladus fluitans for approximately 30 m. The stream then reforms 10 m above the boundary of Eshwood at reach 11.

3.44 Eshwood to River Deerness (reaches 12-19)

At the boundary of the wood, the stream receives several smaller tributaries which contain non acidic water. Here the stream bed is covered with a mixture presumed to consist of a very unstable, friable complex of iron aluminium and organic materials, which precipitate out over an area of about 20 m. The water at this point often supports a considerable growth of fungi and bacteria which are macroscopically obvious. In the winter of 1972 the plantation of deciduous and fir trees was felled and replanted, thus increasing the amount of light available to the organisms in the stream.

At approximately 1.2 km from the source, the stream flows into Redburn, and is quickly diluted out to about pH 7.0. The immediate effect on Redburn is minimised after 15-20 m. Redburn flows for a further 2 km before entering the River Deerness, at Ushaw Moor. During its passage Redburn receives untreated sewage, which in summer causes deoxygenation in the higher reaches, and also non acidic mine drainage from Ushaw Moor colliery in its lower reaches. As the burn receives several different forms of pollution, it is impossible to determine how much the effect seen at its confluence with R. Deerness, is due to the presence of water from Brandon Pithouse Acid Stream.

3.5 Changes to the stream which occured during the study

The general description of the streams given previously has altered at various times during the study period and in some instances these changes have completely altered the characteristics of parts of the stream system.

3.51 Freezing over of the reservoir

During the winter the reservoir has frozen over on several occasions for brief periods.Consequently during these periods water ceased to flow from the reservoir, thus causing the section of the stream below the reservoir to dry up.During periods when this occured the only acid water flowing down the main stream to Redburn originated from stream B. As previously mentioned the pH of stream B was considerably greater than stream A thus leading to relatively large changes in the pH and other chemical parameters in the reaches down stream of the confluence.

3.52 Broken pipe - changes from July 1974 - November 1974

In July 1974 the pipe feeding the reservoir was broken and a considerable amount of acid water flowed into the field.This had two effects, firstly the grass in the field which came in contact with the acid water was destoyed and secondly, after about two weeks the level in the reservoir dropped below the level of the out flow, resulting in the discontinuation of the stream A below the reservoir.Stream B was again the only source of water flowing down the stream below the confluence, except on a few occasions following heavy rainfall which filled the reservoir sufficiently to allow water to flow down the stream.After one month, the pH and acidity gradient down the stream below the confluent altered considerably (see 5.42).The pH was maintained near the pre-July value at reach 10 because of the small acid seepage which enters the stream at this point .However, any increase in flow down the stream reduced the effect of the seepage.

3.53 Changes from November 1974 - March 1975

In November the broken pipe was replaced and the reservoir refilled with acid water.Within a few days of the stream A running its full course the pH of the stream below the conluence at reach 8 returned to its previous value(see 5.5).Immediately, the friable iron precipitate covered the stream bed at and below the confluence where it had largely disappeared in the July-November period.

Shortly after the pipe was repaired,stream B was dredged removing all the <u>Juncus effusus</u> that was growing at the edge of the stream.This action changed the stream from a slow moving, deep ditch to a shallow,faster flowing type.In addition the pH increased from 2.9 to 3.0 -3.5.The reason for the change in pH is not clear.Possibly the reconstruction of the tip in the immediate vicinity may have diverted some acid water away from the ditch, or alkaline water may have been seeping into theditch thus diluting the acidity. In addition to an increase in pH the total discharge from stream B has decreased slightly since dredging took place.This lead to a decrease in pH below the confluence at reach 8 and a consquential decrease in the amount iron oxide precipitate at reach 8 but an increase in precipitate at reach 12 -14.

As previously mentioned in 2.22 because of the above changes the chemical and floristic data are presented in three sections (see Table 2.2)

4. SURVEYS OF WATER AT OR BELOW pH 3.0 IN ENGLAND

4.1 Chemical parameters

The details of the physical and chemical parameters measured at each site during the late summer and late winter surveys are given in Table 4.1 and the maximum, mean and minimum values for all reaches are summarized in Table 4.9.

4.11 pH

The pH values of the reaches sampled ranged from pH 1.5 to 3.0, with a mean value of pH 2.7. The distribution of recorded values within the range can be seen more clearly from Table 4/10. The results show that the distribution over the range was uneven, with only 15 reaches having a pH value of less than 2.5 and the greatest number having a pH of 3.0. The lowest value of pH 1.5 was recorded at site 4, reach 5; this reading was taken after a long period of hot weather and may be partly accounted for by evaporation and the consequential increase of ions in the water.

4.12 Acidity

The acidity of these waters ranged from 64000 mg l^{-1} $CaCO_3$ at site 13, reach 2, to 116 mg l^{-1} $CaCO_3$ at site 9 reach 5. Although measurement of acidity takes into account the total H ion concentration, there was no significant correlation between pH and acidity (see 4.7) over the pH range 1.5-3.0. The lack of correlation suggests that acid salts provided an important contribution to the acidity of the water and where

site no.	name	no. stream complex	total reach no.	O.D. 420 nm A	O.D. 420 nm B	conductivity micro-mhos A	conductivity micro-mhos B	pH A	pH B	acidity as $CaCO_3$ A	acidity as $CaCO_3$ B	PO_4-P A	PO_4-P B	NH_4-N A	NH_4-N B	NO_3-N A	NO_3-N B	SO_4-S A	SO_4-S B	Cl A	Cl B	Si A	Si B	O_2 %saturation A	O_2 %saturation B	temp °C A	temp °C B	current ms^{-1} B
1	Walkmill	1	1	0.042	0.011	4200	3500	2.9	3.0	2080	4100	0.10	0.14	0.60	3.90	<0.5	0.56	1265	1794	27.0	59.1	50.0	53.0	55	60	18.6	15.6	0.23
			2	0.101	0.069	7000	3500	3.0	2.9	3320	3800	0.24	0.15	3.00	2.10	<0.5	0.43	2383	1632	21.0	60.2	67.0	56.0	65	82	12.6	10.8	<0.03
			3p	0.041	0.077	5500	4000	2.9	2.75	2400	7800	0.04	0.88	2.6	3.08	<0.5	0.40	1950	2106	42.0	29.5	69.0	77.0	55	80	15.3	8.3	
2	Oatlands	1	1p		0.146		2750		2.6		6000		0.38		1.80		0.26		1638		33.7		43.5		26		7.4	<0.03
			2		0.246		3700		2.4		8300		0.08		0.79		0.26		1833		35.9		56.0		42		11.3	<0.03
3	Brandon Pithouse	2	1	0.028	0.23	2000	2000	2.6	2.6	1392	1500	0.43	0.26	0.64	0.30	<0.5	0.40	412	312	86.0	27.8	39.0	32.5	85	95	11.3	6.1	0.36
			2	0.025	0.016	1800	1500	2.6	2.6	1428	1500	0.45	0.30	0.46	0.40	<0.5	0.21	385	273	74.0	26.8	35.0	32.0	95	95	9.5	7.0	0.23
			3	0.015	0.007	1800	1500	2.6	2.6	1196	1500	0.17	0.28	0.33	0.42	<0.5	0.31	387	250	52.0	26.9	36.0	31.2	86	98	11.2	7.2	0.20
			4p	0.004	0.006	2500	1750	2.9	2.7	1472	1900	0.01	0.14	0.25	0.55	<0.5	0.32	605	429	23.0	24.6	44.0	37.0	95	96	11.4	7.4	<0.03
4	Rowley	2	1	0.014	0.775	17000	7000	2.3	2.4	13800	12500	7.20	1.64	0.15	1.30	<0.5	2.04	2145	5889	155	128	97.0	77.5	60	85	19.0	8.8	0.12
			2	0.120	0.219	7500	3200	2.3	2.8	3320	5900	1.00	1.36	9.3	0.68	<0.5	0.55	4080	3549	80.0	25.3	55.0	25.0	40	85	20.0	6.5	0.42
			3	0.170	0.440	17500	4700	3.0	3.0	13000	11000	44.0	1.65	10.3	0.80	<0.5	0.37	2028	2340	65.0	141	47.0	43.5	50	87	21.0	7.6	0.26
			4		0.382		4500		2.6		15500		0.28		4.48		0.38		1404		46.4		37.0		80		12.0	<0.03
			5	0.160		20000		1.5		20000		76.0		0.23		<0.5		2730		145		111		30		11.6		
			6	0.140		5500		2.8		2200		1.8		10.5		<0.5		2028		60.0		40.5		40		14.8		
			7	0.650		12500		1.8		10600		1.05		0.66		<0.5		1677		28.0		65.5		40		20.0		
5	Deerplay	1	1	0.600	0.276	3750	3700	2.7	2.6	1656	8300	1.43	1.54	0.73	1.37	<0.5	0.15	4875	3705	28.0	21.1	33.5	50.5	90	48	20.1	7.6	0.25
			2	0.140	0.187	3000	3000	2.7	2.7	1620	6200	1.20	1.60	0.65	1.50	0.51	0.60	5304	1833	27.0	23.2	35.0	53.5	80	85	18.5	7.1	0.36
			3	0.061	0.200	4500	3250	3.0	2.7	1650	5900	0.31	1.56	1.05	1.10	0.50	0.56	3978	1638	32.0	18.9	35.0	46.5	81	92	20.0	6.6	0.27
6	Chisnall Hall	3	1p	0.058	0.117	10000	3250	2.7	2.7	15660	8900	0.05	0.15	2.50	0.82	<0.5	2.15	4485	897	18.0	25.3	28.5	15.0	30	89	20.2	9.1	
			2	0.091	0.112	10000	3250	2.5	2.7	15520	4100	0.05	0.12	2.48	1.44	<0.5	0.65	2535	887	14.0	27.4	4.5	13.5	90	100	19.3	7.4	
			3	0.231	0.685	12000	10000	2.1	2.5	8565	2950	0.86	3.70	2.10	1.78	0.7	2.10	3413	3939	18.0	27.4	6.2	11.0	80	73	20.2	10.1	<0.03
			4	0.090	0.122	6500	5000	2.5	2.5	9200	8600	0.12	0.29	2.40	1.66	0.5	1.53	2730	1131	14.0	23.2	5.5	17.0	90	97	18.6	8.9	<0.03
			5	0.081	0.050	6500	4500	2.5	3.0	8000	3100	0.14	0.05	2.51	1.80	<0.5	0.30	2418	975	31.0	25.3	4.5	38.0	79	60	17.9	8.2	<0.03
			6p	0.065	0.145	6500	4500	2.5	2.8	6560	5500	0.13	0.14	2.38	0.78	<0.5	0.70	2340	702	21.0	29.5	6.0	23.0	84	42	17.3	8.8	<0.03
			7	0.078	0.134	6000	4500	2.5	2.8	5360	4600	0.10	0.25	2.10	0.92	<0.5	2.90	2730	975	25.0	29.5	6.5	24.0	95	98	19.0	8.3	0.29
			8p	0.150	0.005	5000	3000	2.8	3.0	3760	1900	0.05	0.29	2.05	1.10	<0.5	0.38	2145	1014	21.0	29.5	37.5	26.0	88	101	20.0	9.3	<0.03
			9	0.121	0.447	12000	5200	2.8	2.7	7360	7900	0.06	0.57	2.25	1.41	<0.5	0.58	4480	1443	25.0	29.0	24.0	41.0	18	106	21.2	9.6	<0.03
7	Welsh Whittle	1	1	0.042	0.011	2700	4250	3.0	3.0	768	2300	0.05	0.31	0.31	3.10	<0.5	0.40	429	936	40.0	27.4	6.6	25.0	59		8.5		<0.03
8	Gibfield	1	1p		0.091		3000		2.6		4000		0.21		1.41		2.50		2418		29.5		47.0		48		8.5	
			2		0.088		3000		2.6		4000		0.06		1.30		0.30		1090		90.7		50.0		60		7.2	0.46
			3	0.046	0.026	6500	4500	2.7	3.0	2920	4600	0.14	0.06	2.10	3.50	<0.5	0.26	3042	5460	44.0	92.8	44.0	21.0	60	62	10.8	7.1	<0.03
			4		0.097		3500		2.8		3500		0.04		2.00		0.26		3744		50.6		40.5		78		8.5	0.42
9	Denby	2	1	0.061	0.572	13500	8000	2.2	2.9	2224	14100	0.26	0.25	6.75	2.60	<0.5	0.22	4455	3822	27.0	75.9	21.0	26.6	70	85	16.5	7.3	0.23
			2	0.260	0.528	10000	7500	2.0	2.9	16970	13400	0.15	0.35	7.10	2.78	<0.5	0.56	3660	2184	27.0	65.4	29.5	27.5	75	60	18.0	7.6	0.20
			3	0.091	0.490	11500	7500	2.1	2.9	16760	13900	0.15	0.10	3.75	0.82	0.5	0.48	4260	2145	29.0	46.4	26.0	27.0	80	72	15.2	7.4	0.25
			4	0.120	0.208	2500	4000	3.0	3.0	180	7100	0.01	0.02	1.60	1.57	0.8	0.28	685	1365	33.0	37.9	9.0	13.0	85	90	12.3	6.6	0.27
			5	0.430	0.445	9000	600	3.0	2.8	116	13500	0.07	1.90	5.10	0.60	<0.5	0.22	2065	1599	56.0	23.2	20.0	31.5	54	88	13.2	6.8	0.28
			6	0.141	0.543	10000	650	2.5	2.8	13800	13100	0.13	2.10	3.91	2.24	<0.5	3.00	1570	1755	132	23.2	25.0	32.5	68	77	12.4	5.9	<0.03
10	Cannock	1	1p	0.083	0.023	14200	1500	2.1	2.4	4560	5000	0.25	0.82	1.05	1.00	<0.5	0.31	3042	3642	44.0	32.0	44.5	31.0	95	87	10.3	7.4	
11	Polesworth	1	1	0.043	0.042	4700	3000	2.5	2.9	1840	2100	0.10	0.06	2.60	4.00	<0.5	0.38	1028	1209	14.0	130	13.5	11.0	90	97	21.0	7.8	
			2p	0.042	0.041	4000	3000	2.5	2.9	2000	2100	0.08	0.07	2.38	4.18	<0.5	0.26	1250	858	18.0	255	12.8	11.5	90	100	21.0	7.1	
			3	0.041	0.034	4500	3500	2.6	2.9	2000	1800	0.12	0.17	2.41	5.30	<0.5	0.26	1450	936	21.0	175	14.3	10.5	92	90	19.5	7.3	0.12
12	Kingsbury	1	1	0.042	0.014	6500	4500	2.9	3.0	5760	3800	0.13	0.10	1.90	4.36	1.08	0.76	2738	2379	88.0	173	57.0	59.0	37	92	11.6	8.2	<0.03
			2	0.071	0.144	4500	6500	2.9	3.0	3680	5300	0.05	0.03	5.10	4.55	3.2	0.40	2028	3080	149	156	36.5	67.5	43	12	14.8	10.8	<0.03
			3	0.073	0.039	4500	5200	2.9	3.0	3200	4500	0.68	0.06	4.40	5.20	4.0	0.55	1677	1950	170	173	34.5	62.0	80	89	20.0	8.3	0.10
13	Birch Coppice	1	1p	0.431	0.241	7500	4250	2.2	2.6	15200	10000	0.82	1.42	1.50	0.70	<0.5	0.25	4875	2340	151	67.5	37.5	21.5	56	95	20.1	7.4	
			2	0.310	0.786	30000	1500	2.1	2.5	64000	46000	0.58	8.30	10.8	4.36	<0.5	0.50	5304	8580	168	223	35.5	72.0	40	87	18.5	7.8	<0.03
			3p	0.630	0.763	9000	9000	2.3	2.6	16880	20000	0.50	5.60	2.18	3.50	<0.5	2.00	3978	4680	225	80.2	114	45.5	60	92	20.0	7.1	
14	Bridford	1	1	0.046	0.032	1000	1050	3.0	3.0	740	1020	0.10	0.15	0.14	0.30	<0.5	0.24	936	508	29.5	32.1	11.6	26.0	10		14.6		0.21
			2p	0.007	0.008	1300	800	2.9	2.9	740	744	0.08	0.13	0.10	0.36	0.50	0.31	468	507	33.7	26.1	4.6	25.0	70		10.8		
			3	0.004	0.007	1500	1000	2.9	3.0	928	844	0.085	0.13	0.05	0.51	2.90	0.26	429	390	33.7	27.3	7.6	25.0	70		10.7		0.32
15	Industrial Effluent	1	1	0.037	0.005	2000	750	2.9	7.1	580	216	0.12	0.47	0.13	0.60	2.50	2.15	429	468	56.9	67.8	6.3	6.0	52		4.2		
			2	0.037	0.033	1800	750	3.0	7.1	420	220	0.14	0.14	0.50	0.36	1.00	1.23	273	154	132.9	48.5	9.0	7.C	50		4.2		
			3	0.037	0.031	1900	800	3.0	7.1	380	215	0.23	0.21	0.85	0.32	2.25	1.51	429	321	177.3	54.1	8.4	7.3	48		4.2		
16	Dowgang		1		0.031		1100		2.9		412		0.08		0.35		0.22		405		25		5.7		88		10.1	0.16
			2		0.120		1200		3.0		404		0.26		0.36		0.31		355		17.5		8.7		92		11.3	0.26
			3		0.121		1150		3.0		384		0.24		0.32		0.30		252		52.5		7.5		94		11.3	0.27

site no.	name	no. stream complex total	reach no.	Na A	Na B	K A	K B	Mg A	Mg B	Ca A	Ca B	Zn A	Zn B	Cu A	Cu B	Mn A	Mn B	Fe A	Fe B	Al A	Al B	Pb A	Pb B	Co A	Co B	Ni A	Ni B
1	Walkmill	1	1	169	130	14.5	15.5	551	390	556	389	0.96	0.85	0.023	0.07	45.2	34.2	193	225	149	119	0.010	0.21	1.85	1.31	2.78	4.52
			2	785	621	5.61	3.14	780	678	510	410	1.10	1.20	0.24	0.12	88.7	72.4	70.0	111	325	206	0.018	0.10	2.48	2.31	3.32	4.11
			3p	45.3	62.8	7.80	5.00	682	545	520	435	1.40	1.29	1.24	2.32	113	76.9	105	255	411	400	0.020	0.32	2.62	2.15	3.84	6.21
2	Oatlands	1	1p		7.9		0.81		29.0		272		0.41		2.10		5.1		480		149		0.17		2.15		2.53
			2		7.9		0.39		76.0		420		0.71		2.67		8.7		810		295		0.22		1.19		6.52
3	Brandon Pithouse	2	1	12.3	11.2	0.57	0.42	68.3	77.0	64.2	60.1	1.14	0.97	0.69	0.65	6.6	6.3	110	95.0	31.2	32.0	0.013	0.050	0.20	0.21	0.48	1.30
			2	12.6	12.0	0.74	0.57	66.4	80.1	67.8	57.2	1.10	0.96	0.63	0.63	7.1	6.3	100	90.0	31.9	30.5	0.012	0.050	0.008	0.08	0.05	1.21
			3	13.2	13.0	1.94	0.31	65.7	82.0	72.0	58.6	1.07	0.95	0.57	0.63	6.9	6.2	85.0	80.0	29.0	30.1	0.014	0.040	0.007	0.04	0.03	1.14
			4p	119	76.5	7.60	5.00	152	150	148	133	1.36	1.50	0.36	0.48	9.1	10.6	21.0	36.0	29.1	38.5	0.005	0.080	0.03	0.23	0.04	0.80
4	Rowley	2	1	455	77.5	2.55	0.39	920	325	490	433	10.2	4.38	3.58	4.40	160	54.4	4800	2500	1570	668	0.33	0.37	4.05	1.52	7.45	5.80
			2	380	106	2.50	2.50	715	110	405	176	3.79	1.59	0.96	0.66	126	29.3	4200	230	320	87.5	0.095	0.13	0.84	0.41	2.24	2.22
			3		132		2.20		240		256		2.86		1.30		48.1		1400		294		0.25		0.85		3.43
			4	500	358	3.40	12.2	324	535	428	361	4.79	3.92	0.08	0.45	60.0	84.8	420	3650	280	330	0.034	0.43	1.68	1.10	3.26	5.71
			5	405		0.31		1510		480		17.1		8.86		285		4950		2500		0.116		5.82		11.3	
			6	77.0		1.35		116		400		2.74		0.74		55.0		454		152		0.064		0.83		1.20	
			7	1250		9.25		905		420		7.41		2.87		151		345		890		0.057		2.48		4.64	
5	Deerplay	1	1	17.8	17.3	0.67	1.20	105	153	300	415	2.55	4.33	1.24	3.19	27.4	31.5	431	840	94.5	146	0.007	0.17	0.76	1.00	2.72	4.20
			2	19.5	26.2	0.82	0.89	100	120	260	258	2.67	4.64	1.26	2.84	28.7	42.4	420	610	100	176	0.003	0.17	0.86	1.12	2.84	4.51
			3	40.0	23.3	1.80	0.66	23.8	130	383	249	4.54	4.99	0.54	3.02	89.0	40.8	390	590	190	212	0.015	0.19	2.15	1.30	3.30	4.32
6	Chisnall Hall	3	1	581	159	15.8	2.90	720	340	399	164	1.09	0.79	0.18	0.38	85.0	22.9	1310	275	169	101	0.026	0.15	2.08	0.72	3.04	2.23
			2p	392	155	1.42	2.80	724	330	236	157	0.61	0.75	0.13	0.36	41.4	21.7	353	260	142	101	0.012	0.10	1.06	0.71	1.90	3.31
			3	230	40.9	1.00	0.14	810	475	520	437	1.32	2.97	3.10	16.0	40.1	26.1	1680	4700	400	836	0.21	0.48	2.34	3.20	8.41	12.5
			4	395	173	1.35	2.40	730	368	240	165	1.16	0.86	0.29	0.51	41.9	22.7	371	315	142	109	0.005	0.14	1.28	0.72	2.03	3.10
			5	385	319	1.47	14.1	766	575	237	311	0.50	1.38	0.14	0.36	41.2	40.2	370	155	141	72.0	0.013	0.16	1.25	0.82	1.95	3.32
			6p	402	209	3.40	8.40	755	595	250	221	0.61	1.12	0.13	0.81	42.0	31.5	357	415	132	126	0.013	0.17	1.20	1.00	1.88	3.91
			7	403	231	3.50	8.20	717	575	248	215	0.62	1.10	0.13	0.80	41.5	33.2	352	400	132	126	0.007	0.17	1.25	1.01	1.89	3.26
			8p	180	51.7	21.2	16.5	870	505	377	397	0.54	0.77	0.14	0.22	40.6	28.9	155	10.1	81.3	55.0	0.008	0.18	1.23	0.55	1.88	3.05
			9	278	53.7	52.8	16.0	398	425	389	425	0.32	1.16	0.14	0.31	77.0	81.0	679	1250	35.0	130	0.017	0.34	1.25	1.21	1.02	5.50
7	Welsh Whittle	1	1	13.6	166	16.0	24.7	36.0	620	138	453	0.09	0.58	0.08	0.12	0.35	11.5	12.5	25.0	23.0	36.5	0.24	0.21	0.05	0.30	0.05	2.01
8	Gibfield	1	1p		82.5		4.30		110		379		3.80		0.68		11.2		265		71.5		0.17		2.71		6.50
			2		87.6		4.10		133		450		3.75		0.87		11.4		270		74.5		0.17		0.41		2.70
			3	738	603	11.1	13.6	350	310	400	336	0.96	2.50	0.42	0.04	23.5	34.0	1020	535	106	73.5	0.016	0.22	0.13	0.40	1.15	2.33
			4		227		7.70		193		432		3.01		0.59		15.8		365		76.0		0.20		0.55		2.52
9	Denby	2	1	690	518	13.2	19.9	144	930	444	415	6.70	5.40	0.11	0.24	192	100	4900	3250	179	149	0.083	0.42	2.94	1.61	4.28	7.54
			2	505	358	4.4	13.8	980	805	426	391	5.71	7.60	0.06	0.09	133	96.1	2700	2600	154	201	0.040	0.36	1.65	1.81	3.91	8.05
			3	585	395	1.65	21.7	1220	905	445	420	6.40	6.81	0.11	0.15	163	100	3600	2800	172	173	0.057	0.39	2.08	1.82	4.05	7.03
			4	640	141	1.08	7.50	122	340	81.0	175	0.76	2.60	0.02	0.11	16.4	38.8	290	770	19.5	82.5	0.006	0.13	0.43	0.71	0.45	3.80
			5	530	233	33.0	6.00	925	565	415	295	5.50	6.12	0.05	0.69	128	83.3	2900	1925	114	306	0.077	0.32	1.40	1.70	3.34	6.71
			6	550	211	29.0	3.70	1125	640	435	340	5.90	5.80	0.08	0.63	132	79.1	2750	1725	124	292	0.096	0.30	1.52	1.71	3.50	6.28
10	Cannock	1	1p	11.4	23.6	4.40	5.20	205	278	338	401	88.0	62.0	3.10	3.21	114	120	23000	10000	425	501	0.080	0.061	3.20	4.10	5.10	3.21
11	Polesworth	1	1	385	329	5.00	6.50	284	208	348	265	0.85	0.72	0.018	0.03	50.1	45.4	83.0	105	4.86	3.72	0.020	0.10	0.80	0.41	0.63	2.52
			2p	378	330	5.60	7.23	206	195	330	249	0.83	0.71	0.019	0.026	51.0	45.2	85.0	110	4.76	3.72	0.001	0.13	0.81	0.40	0.56	2.01
			3	330	318	5.12	6.32	210	190	325	239	0.86	0.64	0.017	0.026	50.0	44.4	76.2	95.0	4.83	3.92	0.012	0.23	0.80	0.41	0.42	1.91
12	Kingsbury	1	1	690	608	10.3	10.8	334	335	525	435	24.6	41.4	0.052	0.035	112	99.8	186	23.5	169	138	0.013	0.22	2.04	2.10	4.68	4.60
			2	440	720	3.40	3.00	268	428	303	425	18.0	22.9	0.14	0.28	67.2	100	112	465	111	175	0.019	0.25	0.92	2.21	2.48	5.93
			3	395	623	3.50	9.10	246	323	290	359	20.5	37.9	0.13	0.041	62.2	103	96.0	120	100	138	0.013	0.26	1.66	1.91	2.00	5.31
13	Birch Coppice	1	1p	213	151	0.07	0.25	260	163	232	130	9.74	7.8	2.51	1.56	53.0	36.9	809	660	96.0	620	0.016	0.23	6.00	2.20	9.70	7.62
			2	856	942	0.92	1.57	379	480	344	398	76.5	32.1	4.60	2.70	110	106	9450	8000	3130	2000	0.019	0.91	20.0	11.30	50.4	27.0
			3p	3550	1640	0.05	6.00	2340	1310	385	298	8.48	14.9	2.90	2.60	544	211	1702	2400	775	656	0.29	0.50	6.80	5.50	11.4	14.0
14	Bridford	1	1	8.4	20.4	8.40	7.70	28.6	31.5	68.0	73.3	54.5	67.4	0.006	0.141	29.9	33.0	112	132	4.7	5.1	1.80	1.90	0.19	0.23	0.53	2.12
			2p	8.0	8.2	8.00	1.60	28.3	6.60	67.0	4.0	193	117	2.37	1.88	44.8	21.5	18.5	7.5	10.4	6.7	0.95	1.29	0.29	0.33	0.86	0.81
			3	3.9	20.1	3.90	7.40	25.2	30.8	61.1	72.0	57.0	64.0	0.104	0.088	31.0	32.5	34.5	91.5	5.8	12.6	1.52	1.75	0.18	0.28	0.53	1.02
15	Industrial Effluent	1	1	12.5	35.0	12.5	5.9	20.2	16.3	136.0	123	0.21	0.009	0.125	0.008	0.36	0.02	15.8	0.26	5.7	6.0	0.20	0.051	0.05	0.21	0.09	0.56
			2	21.5	29.3	21.0	5.7	37.5	14.7	122.0	123	0.33	0.013	0.067	0.009	12.7	0.02	42.4	0.33	26.8	10.3	0.045	0.27	0.27	0.23	0.53	0.53
			3	16.1	25.1	16.4	5.6	34.3	15.1	147.6	122	0.076	0.012	0.078	0.008	0.34	0.02	11.3	0.31	2.32	5.3	0.22	0.042	0.04	0.23	0.05	0.54
16	Dowgang		1		4.30		1.78		23.1		6.60		0.35		0.040		3.91		31.0		3.78		0.042		0.08		0.05
			2		4.15		1.94		106		32.7		2.27		0.102		9.80		99.0		12.2		0.016		0.33		0.18
			3		4.00		1.74		107		29.6		2.08		0.092		9.10		88.0		11.1		0.053		0.28		0.18

there were high acidity values, but not extremely low pH values, the environment may be more restricting to the species than indicated by the pH value.

4.13 Heavy metals

The results show that besides having a low pH, the acid mine drainage waters from the coal mines contained large concentrations of one or more heavy metals. At least one of the metals studied was in at a concentration of more than 1.1 mg l^{-1} on one or other sampling days. However, instances were recorded for each element where its concentration was relatively low. Zn was generally found to be the most abundant heavy metal, with a mean value of 16.0 mg l^{-1} and a maximum concentration of 193 mg l^{-1} at site 14 reach 2. Large concentrations of Cu, Co and Ni were also recorded in many reaches with maximum values of 16.0, 20.0 and 50.4 mg l^{-1} respectively. Water from site 13, reach 2 had the largest overall heavy metal loading, although individual elements were recorded at higher levels in other reaches. This reach also had the highest acidity value recorded, but not the lowest pH value. The levels of Pb were generally low, compared with the other heavy metals examined. The maximum value of Pb was 1.90 mg l^{-1} and the minimum 0.001 mg l^{-1}. As veins of lead are not usually associated with coal mining areas, it was not surprising to find low values of this element in the water.

4.14 Fe, Al, Mn

The concentrations of these elements were also very large compared with normal streams, as demonstrated by their mean values (Fe 1111 mg l^{-1}; Al 258 mg l^{-1}; Mn 64 mg l^{-1}). The occurrence of large concentrations of iron was invariably associated with high Al and to a lesser extent, high Mn concentrations and for this reason Fe has been included in this section rather than the other heavy metals (4.13).

4.15 Na, K, Ca, Mg

The waters ranged considerably in the degree of hardness, but were generally considered to be soft. The lowest Mg and Ca levels of 6.6 and 4.0 mg l^{-1} were recorded for site 14 in survey A. The concentrations of the cations measured were not particularly noteworthy, except for the K level at site 13, reach 3 which was 0.05 mg l^{-1}.

4.16 Optical density and Conductivity

The optical density values of some waters were unusually high, but this was not surprising in waters where large concentrations of Fe and Al were recorded. The large iron values often produced bright red water, as well as a red-orange precipitate. This was most obvious at sites 3 and 13. The large conductivity values were also expected in water containing such large concentrations of ions. Conductivity may be considered indicative of the total ion status of acidic waters and there was significant correlation between conductivity and many of the ions which occurred in large amounts.

4.17 PO_4-P, NH_4-N, NO_3-N

The concentrations of these nutrients were often at a sufficiently high level as to be considered indicative of a moderately eutrophic environment. In the late summer survey 56% of the reaches and 61% of those in the winter survey had both PO_4-P levels 0.1 mg l^{-1} and combined inorganic N levels of 1.0 mg l^{-1}. However the results for a few reaches were considerably lower than those values, for example, site 9, reach 4; PO_4-P 0.01 and 0.02 mg l^{-1} and site 4, reach 1 N 0.65 mg l^{-1}. The total concentration of N present in the acidic environment was mainly contributed by the NH_4-N, as NO_3-N levels were generally less than 0.5 mg l^{-1}.

4.18 Si, Cl, SO_4-S

The concentrations of Si and SO_4-S were always high, (mean values of 36.5 mg Si l^{-1} and 2600 mg S l^{-1}) and in some instances very high (maximum values: 114 mg Si l^{-1} and 8600 mg S l^{-1}0). The concentrations of Cl were not unusually high, as indicated by a maximum value of 114 mg l^{-1} and a minimum value of 14.0 mg l^{-1}.

It is unlikely that the diatom populations would have been limited at any time by Si, although nothing is known of the availability of the Si at such low pH values. It is probable that any buffering action which exists at these low pH values would involve salts containing these anions.

4.19 Percentage oxygen saturation

The concentration of dissolved oxygen was generally low for organically unpolluted waters, particularly when the large growth of algae which sometimes occurred, was taken into account. A mean value of 70% and a minimum value of 10% were recorded in both surveys. The reason for the very low value was thought to be due to continued oxidation of ferrous to ferric ion after the water had issued from the ground. This occurred at site 14, reach 1, where the oxygen increased from 10% to 70% as the pH decreased from 3.0 to 2.9. It was assumed that H_2SO_4 was still being produced above ground.

4.2 Physical parameters

4.21 Temperature

The temperature of these waters was consistent with normal streams for the time of year sampled, except where water was issuing from a burning tip. At these sites, quite high values were recorded, even when the air temperature was near freezing, for example at site 1, reach 1 the water temperature at the source of the stream was 15^{o}C. Although these springs may be considered warmer than normal underground springs, there were no examples of thermal acid streams.

4.22 Current speed

As already mentioned in 2.31, current speed was measured only during the second survey. The results, expressed as the maximum current speed per reach, ranged from values greater than 0.03 to 0.46 ms^{-1} with 0.03 being the lowest speed the meter could measure. The current speed sometimes varied considerably within the 10 m reach sampled and therefore, it was considered most useful to record the maximum value any alga would have to withstand, in order to survive in a given 10 m reach. Where no result was given for a reach, this indicates that either the reach was a pool habitat, or else lack of access prevented a reading from being taken.

4.3 Comparison of the physical and chemical data between the two surveys

A comparison of the data collected for individual reaches demonstrates how chemically variable the acidic environment can be, even when two reaches in the same complex are considered. As well as variability between different reaches, there was frequently a marked difference between values obtained at different times of the year. A comparison of the two surveys is given in Table 4.2 and shows that 10 parameters were greater in the summer than the winter surveys and 13 parameters were greater in the winter. As the volumes of water in the streams were lower in the late summer than late winter, it was not surprising that some parameters were higher in the late summer survey. However, the values obtained for 13 parameters did fall the other way by a sufficiently large amount as to indicate that straightforward dilution was not affecting these parameters as would be expected. The fact that levels of Na, Mg, Ca, Mn and hydrogen ion concentration were lower in winter, could be attributed to dilution by higher flows, but all of the heavy metals, acidity and Al were higher in winter than summer. It was thought that both dilution and a decrease in the amount of acid produced accounts for the higher pH values in winter (see 8.23).

Table 4.2 Comparison of values for physical and chemical parameters collected in late summer and winter surveys.

	no. greater in late summer (A) than winter (B)	no. greater in winter than late summer	no. same on both surveys
OD_{420} nm	17	26	0
conductivity (micro-mhos)	34	6	3
pH	8	24	11
acidity (as $CaCO_3$)	18	25	0
Na	32	11	0
K	17	25	1
Mg	26	17	0
Ca	30	13	0
Zn	19	24	0
Cu	11	31	1
Mn	30	13	0
Fe	19	24	0
Al	22	21	0
Pb	1	42	0
Co	22	21	0
Ni	8	35	0
PO_4-P	14	29	0
NH_4-N	24	19	0
NO_3-N		16	
SO_4-S	27	16	0
Cl	18	25	0
Si	17	26	0
O_2, %	9	29	1
Temp °C	43	0	0

4.4 Photosynthetic organisms recorded at or below pH 3.0

4.41 Species composition

A total of 28 species of photosynthetic organisms were found growing in water at or below pH 3.0 (Tables 4.3 and 4.4). It was possible to give binomials to 21 of the species recorded. Species represented only by individual cells, or filaments which had almost certainly been washed in from different habitats, were not included. Although several of the species listed were recorded on one occasion only, they were both obviously healthy and in sufficient numbers to justify their inclusion as part of the stream flora. In addition to the designated sampling reaches, the spot samples (see 2.52) that were taken from other areas of the streams failed to show any species not present in the reaches.

From Table 4.4 it can be seen that _Euglena_ _mutabilis_ (90%) _Pinnularia_ _acoricola_ (71%) and _Gloeochrysis_ _turfosa_ (61%) were considerably more widespread than the other species. _Nitzschia_ _subcapitellata_, _Nitzschia_ _ellipitica_ var. _alexandrina_, _Eunotia_ _exigua_, _Chlamydomonas_ _applanata_ var. _acidophila_ and _Hormidium_ _rivulare_ were also relatively common, occurring in over 20% of all possible reaches.

In both surveys diatoms were represented by 11 species, making them the largest single phylum represented. The mosses, _Drepanocladus_ _fluitans_ and _Dicranella_ sp. occurred both in the protonemal and in the adult forms. Unlike most habitats where mosses grow, the protonema occurred more frequently than the adult, particulary for _Dicranella_ sp.

Table 4.3 Summary of photosynthetic organisms found in England at ≤ pH 3.0

	algae	moss	flowering plants
streams associated with mining	19	2	1
pools only	4	0	1
steams from industrial effluent	1	0	0
total	24	2	2 =28

species	author	present in survey A (out of 46 reaches)	present in survey B (out of 49 reaches)	present in A + B (out of 95 reaches)	present in either A or B (out of 52 reaches)	present in either A or B as %
Euglena mutabilis	Schmitz	40	42	82	47	90
Pinnularia acoricola	Hustedt	27	23	50	37	71
Gloeochrysis turfosa	(Pasch.) Bourr.	27	17	44	32	61
Nitzschia subcapitellata	Hustedt	14	14	28	17	32
Nitzschia elliptica var. alexandrina	Hustedt Cholnoky	4	13	17	16	31
Eunotia exigua	(Bréb.) Grun.	12	11	23	14	27
Chlamydomonas applanata var. acidophila	Pringsheim, Fott	13	2	15	13	25
Hormidium rivulare	Kutz.	10	7	17	12	23
Zygogonium ericetorum	Kutz.	8	8	16	10	19
Dicranella sp. (protonema)	(Hedw.) Brid.	6	5	11	7	13
Characium sp.		5	2	7	7	13
Lepocinclis ovum	(Ehr.) Lemm.	0	6	6	6	11
Chlamydomonas sp.		1	4	5	6	11
Dicranella sp.	(Hedw.) Brid.	3	3	6	3	6
Drepanocladus fluitans (adult)	(Hedw.) Warnst.	3	3	6	3	6
Stichococcus bacillaris	Näg.	3	1	4	3	6
Nitzschia sp. type A		3	0	3	3	6
Cryptomonas sp.		1	1	2	2	4
Nitzschia ovalis	Arnott	2	0	2	2	4
Pinnularia microstauron	Ehr.	1	1	2	1	2
Nitzschia palea	(Kütz.) W. Smith	1	1	2	1	2
Navicula sp.		1	1	2	1	2
Drepanocladus fluitans (protonema)	(Hedw.) Warnst.	1	1	2	1	2
Nitzschia sp. type B		1	0	1	1	2
Navicula nivalis	Ehr.	1	0	1	1	2
Ulothrix zonata	Kütz.	0	1	1	1	2
Microthamnion strictissimum	Rabenh.	1	0	1	1	2
Typha latifolia	Lin.	1	1	2	1	2
Juncus effusus	Lin.	1	1	2	1	2

The two angiosperms, Typha latifolia and Juncus effusus, occurred in separate single reaches. However, dead Typha latifolia was also found at site 6, reach 1.

4.42 Comparison of species composition between surveys

The overall floristic composition of the streams showed little difference between the two surveys in contrast to the chemistry (see Table 4.4) The number of occurrences did however vary considerably in the case of a few species. Lepocinclis ovum and Ulothrix zonata were the only species not recorded in the late summer, while Nitzschia sp. type A, Nitzschia sp. type B, Navicula nivalis and Microthamnion strictissimum were not recorded in the winter survey.

In addition, Gloeochrysis turfosa, Hormidium rivulare and Chlamydomonas applanata were much more common in late summer, in contrast to Nitzschia elliptica var. alexandrina which was more widespread in winter. These species seemed to demonstrate a more obvious seasonal trend than many of the other commonly occurring species (see Chapter 7).

4.43 Comparison of habitat type

Of the species recorded, 5 were present only in streams, and 6 only in the pool habitat (Table 4.5) All other species were present in both habitats, although some species were more common in one or the other (Table 4.7). For example, the chlamydomonads and Lepocinclis ovum were more common in pools. Further details of the preferences of the individual species to habitat type are given in Chapter 7. The algal species that were recorded only in pools were all diatoms, none of species present in streams, pools, streams and pools

Euglena mutabilis

Pinnularia acoricola

Gloeochrysis turfosa

Nitzschia subcapitellata

Nitzschia elliptica var. *alexandrina*

Eunotia exigua

Chlamydomonas applanata var. *acidophila*

Hormidium rivulare

Zygogonium ericetorum

Dicranella sp. (protonema)

Characium sp.

Lepocinclis ovum

Chlamydomonas sp.

Dicranella sp. (adult)

Drepanocladus fluitans

Stichococcus bacillaris

Nitzschia sp. type A

Cryptomonas sp.

Nitzschia ovalis

Pinnularia microstauron

Nitzschia palea

Navicula sp.

Drepanocladus fluitans (protonema)

Nitzschia sp. type B

Navicula nivalis

Ulothrix zonata

Microthamnion strictissimum

Typha latifolia

Juncus effusus

species present only in streams

Dicranella sp. (adult)

Cryptomonas sp.

Drepanocladus fluitans (protonema)

Ulothrix zonata

Microthamnion strictissimum

species present only in pools

Nitzschia ovalis

Nitzschia sp. type B

Navicula sp.

Pinnularia microstauron

Typha latifolia

Juncus effusus

which were common, even though there were 12 major pool habitats recorded in the surveys. Both species of flowering plants were restricted to the pool habitat, although *Juncus effusus* has since been found at around pH 3.0 in running water in reach 10 and 11 of Brandon Pithouse Acid Stream.

4.44 Estimation of percentage cover

Where it was possible, an estimation was made of the percentage of a 10 m reach, covered by an organism, or group of organisms. The results are given in Table 4.6 for species which were easily recognizable macroscopically. The identity of the alga covering the substratum was checked by microscopic examination of samples taken from the area. Most reaches had macroscopically obvious growth of plant material on one or both of the sampling days.

As can be seen from Table 4.6 *Euglena mutabilis* was not only the most widespread species (Table 4.4) but also often the most abundant species when viewed macroscopically, sometimes covering as much as 80% of the substratum. The results suggest that this species was as abundant in late winter as it was in late summer and it was estimated to cover at least 10% substratum in 35% of the reaches in the late summer survey and 47% of the late winter reaches. These values may be an under estimation of the actual figure because the alga tended to migrate into the silt in order to avoid high light intensity (see 7.2).

It was impossible to distinguish macroscopically between the different diatom species, therefore, they were considered

Table 4.6 The percentage cover by macroscopically recognizable organisms for each 10 m reach in survey A and B.

			Euglena mutabilis		Diatom species		*Hormidium rivulare*		*Zygogonium ericetorum*		*Campylopus flexuosus* (protonema)		*Gloeochrysis turfosa*	
			A	B	A	B	A	B	A	B	A	B	A	B
1	Walkmill	1	20	60										
		2	5	1					5	10				
		3	30	1	30		5		10	1				
2	Oatlands	1		1										
		2		10		60								
3	Brandon Pithouse	1	40	20										
		2	1	1	10	5								
		3	5	5										
		4					30	15						
4	Rowley	1	70	70		30								
		2	5	1		5								
		3		10		5								
		4	80	80										
		5	10											
		6	1										1	
		7	1										3	
5	Deerplay	1	1										50	
		2	1	5										5
		3	20	1							1			
6	Chisnall Hall	1	2	70										
		2		10										
		3		80										
		4		30		50								
		5		30										
		6		20										
		7	5	10	10									
		8			10	70								
		9	1	60										
7	Welsh Whittle	1							20	70	3	5		
8	Gibfield	1		40		10				40				
		2		1										
		3					30	40	70	50				
		4		1										
9	Denby	1	50	1										
		2	70	80										
		3	40	20										
		4	3	1			1							40
		5	70	20										5
		6	80	45										
10	Cannock	1	80	70	1									
11	Polesworth	1			40	2					30	10		
		2									40	60		
		3									10	60		
12	Kingsbury	1	1			20	10	60						
		2	1	60										
		3	10			10	5							
13	Birch Coppice	1	1											
		2	10	80										
		3		1	1									
14	Bridford	1	1				15							
		2	1		1						1			
		3												

as one organism for the purpose of percentage cover estimations. As a group they were capable of covering up to 70% of the substratum with a film of cells. The filamentous algae and moss protonema at times covered large areas of the streams, but it seemed that these organisms took a longer period to become established and form large areas of cover. Gloeochrysis turfosa was quite frequently macroscopically obvious, but rarely covered large areas of a given 10 m reach.

4.45 Relative abundance

In addition to estimating percentage cover for the 10 m reach, the relative abundance of the species within a 100 mm^2 sampling area was also determined (see 2.53). Table 4.7 gives the greatest relative abundance value recorded for the species at each reach and is the highest value selected from a minimum of five 100 mm^2 samples per reach. This Table also demonstrates the distribution by reach of the individual species.

From Table 4.7 it was possible to estimate the abundance of one species relative to another for all the individual reaches and by doing so, to estimate the occurrence of the species as a dominant or co-dominant organism in the acid environment. Although these data are highly subjective, they do confirm the macroscopic observations and give some idea as to whether an organism is resistant to a particular set of conditions, or whether it merely tolerates the conditions.

Table 4.8 details the number of times a particular score on the 1-5 scale was given to each species and also the number of times it occurred as the dominant species. For a

site no.	name	reach no.	pH A	pH B	Euglena mutabilis A	Euglena mutabilis B	Pinnularia acoricola A	Pinnularia acoricola B	Gloeochrysis turfosa A	Gloeochrysis turfosa B	Nitzschia subcapitellata A	Nitzschia subcapitellata B	Nitzschia elliptica var. alexandrina A	Nitzschia elliptica var. alexandrina B	Eunotia exigua A	Eunotia exigua B	Chlamydomonas applanata var. acidophila A	Chlamydomonas applanata var. acidophila B	Hormidium rivulare A	Hormidium rivulare B
1	Walkmill	1	2.9	3.0	5	5		3	5		2	2		3						
		2	3.0	2.9	5	3	2		4		3	3	3				4			
		3	2.9	2.7	5	5	2		5	2	2								2	2
2	Oatlands	1		2.6		5								5						
		2		2.4		5		2												
3	Brandon	1	2.6	2.6	5	5			5	1					5	3	2		2	1
	Pithouse	2	2.6	2.6	3	2			5	2				5	5	1				
		3	2.6	2.6	5	5														
		4	2.9	2.7	3	4			4		5	2			5	4			5	5
4	Rowley	1	2.3	2.4	5	5	2	5	1								1			
		2	2.3	2.8	5	5	5	5	2	4										
		3		3.0		5		1												
		4	3.0	2.6	5	5	5													
		5	1.5		5		5													
		6	2.8		5				5						1					
		7	1.8		5		4		2								2			
5	Deerplay	1	2.7	2.6	5	1		1	5											
		2	2.7	2.7	5	5		4	2	5					1	1				
		3	3.0	2.7	5	5	1		5	5					2	1				
6	Chisnall	1	2.7	2.7	5	5	2		1	5										
	Hall	2	2.5	2.7	5	5	2	1	2		1	1								
		3	2.1	2.5	5	5	2					1								
		4	2.5	2.5	5	5	3	1	1		3			5						
		5	2.5	3.0	2	5	4		5											
		6	2.5	2.8		5	1		1		3								5	
		7	2.5	2.8	5	5	2	1												
		8	2.8	3.0			2	2	3		4	5		2	2		2			
		9	2.8	2.7	5	5	1	1		2										
7	Welsh Whittle	1	3.0	3.0	5	2	2				2	3								
8	Gibfield	1		2.6		5		5		1				3						
		2		2.6		5														
		3	2.7	3.0	3	4		1	2	1									4	5
		4		2.8		5		5		2										1
9	Denby	1	2.2	2.9	5	5			1											
		2	2.0	2.9	5	5	4	2	1			2		5		2				
		3	2.1	2.9	5	5	4										1			
		4	3.0	3.0	5	5											3		3	
		5	3.0	2.8	5	3				5							5			
		6	2.5	2.8	5	5				3							2			
10	Cannock	1	2.1	2.4	5	5	2													
11	Polesworth	1	2.5	2.9			5	1	4	1	3	2		2	2	5	1			1
		2	2.5	2.9			4	3				4		3	3	2				
		3	2.6	2.9			5	3	3	2	4	4		1	4	5				
12	Kingsbury	1	2.9	3.0	5	4	4	5	1	3	3	1		1					5	5
		2	2.9	3.0	5	5		1	4	5	1	3		2					5	
		3	2.9	3.0	5		1	5				2	1	1					5	
13	Birch Coppice	1	2.2	2.6													5	5		
		2	2.1	2.5	5	5	4													
		3	2.3	2.6	5	5		5												
14	Bridford	1	3.0	3.0	5	5			4										5	
		2	2.9	2.9	5	3									2	5	4	1		
		3	2.9	3.0					3	5			1		3	5	2			
15	Industrial effluent (see text)																			
16	Dowgang	1		2.9		5				3						4				
		2		3.0		5										4				
		3		3.0		5				2						3				

site no.	name	reach no.	Zygogonium ericetorum A	Zygogonium ericetorum B	Characium sp. A	Characium sp. B	Lepocinclis ovum A	Lepocinclis ovum B	Chlamydomonas sp. A	Chlamydomonas sp. B	Stichococcus bacillaris A	Stichococcus bacillaris B	Nitzschia sp. type A A	Nitzschia sp. type A B	Cryptomonas sp. A	Cryptomonas sp. B	Nitzschia ovalis A	Nitzschia ovalis B	Pinnularia microstauron A	Pinnularia microstauron B	Nitzschia palea A	Nitzschia palea B
1	Walkmill	1	5	5																		
		2	5	5									3									
		3	5	5	3			1	2		3		2									
2	Oatlands	1																				
		2																				
3	Brandon	1			2						2	1										
	Pithouse	2													2							
		3																				
		4																			2	1
4	Rowley	1																				
		2																				
		3																				
		4			1																	
		5			2																	
		6																				
		7									1											
5	Deerplay	1																				
		2																				
		3																				
6	Chisnall	1	5					1														
	Hall	2	5	2				4														
		3																				
		4						2														
		5																				
		6						4		2												
		7								3												
		8								1							1					
		9								5												
7	Welsh Whittle	1	5	5																		
8	Gibfield	1		5				3														
		2																				
		3	5	5																		
		4		1																		
9	Denby	1																				
		2																				
		3																				
		4																				
		5																				
		6																				
10	Cannock	1																				
11	Polesworth	1											2				1		1	1		
		2																				
		3														2						
12	Kingsbury	1																				
		2				1																
		3				4																
13	Birch Coppice	1																				
		2																				
		3																				
14	Bridford	1																				
		2	3		4																	
		3																				
15	Industrial effluent (see text)																					
16	Dowgang	1																				
		2																				
		3																				

site no.	name	reach no.	Navicula sp. A	Navicula sp. B	Nitzschia sp. type B. A	Nitzschia sp. type B. B	Navicula nivalis A	Navicula nivalis B	Ulothrix zonata A	Ulothrix zonata B	Microthamnion strictissimum A	Microthamnion strictissimum B	Dicranella sp. (adult) A	Dicranella sp. (adult) B	Dicranella sp. (protonema) A	Dicranella sp. (protonema) B	Drepanocladus fluitans (adult) A	Drepanocladus fluitans (adult) B	Drepanocladus fluitans (protonema) A	Drepanocladus fluitans (protonema) B	Typha latifolia A	Typha latifolia B	Juncus effusus A	Juncus effusus B
1	Walkmill	1											5	4										
		2																						
		3													3	2								
2	Oatlands	1																						
		2																						
3	Brandon	1															5	5	5	5				
	Pithouse	2																						
		3																						
		4															5	5					5	5
4	Rowley	1																						
		2																						
		3																						
		4																						
		5																						
		6																						
		7																						
5	Deerplay	1																						
		2											4	5										
		3					2								4									
6	Chisnall	1																						
	Hall	2																						
		3																						
		4																						
		5																						
		6																						
		7																						
		8																						
		9													5									
7	Welsh Whittle	1																						
8	Gibfield	1																						
		2																						
		3																						
		4																						
9	Denby	1																						
		2																						
		3																						
		4																						
		5																						
		6																						
10	Cannock	1																						
11	Polesworth	1	1	1	2										5	5					5	5		
		2													5	5								
		3											5	4			5	5						
12	Kingsbury	1							1	1					2									
		2																						
		3																						
13	Birch Coppice	1																						
		2																						
		3																						
14	Bridford	1																						
		2													5	3								
		3																						
15	Industrial effluent (see text)																							
16	Dowgang	1													4									
		2																						
		3													3 5									

species	5 A	5 B	4 A	4 B	3 A	3 B	2 A	2 B	1 A	1 B	% occurrence as dominant or co-dominant organism A (46 reaches)	% occurrence as dominant or co-dominant organism B (49 reaches)
	abundance value											
Euglena mutabilis	35	35		3	4	3	1	2		1	76.1	71.4
Pinnularia acoricola	5	7	7	1	1	3	10	3	4	1	10.8	14.3
Gloeochrysis turfosa	7	6	5	1	3	2	5	4	7	4	15.2	12.3
Nitzschia subcapitellata	2	1	2	2	5	3	2	6	3	2	4.3	2.0
Nitzschia elliptica var. *alexandrina*		4			1	3	1	3	2	3		8.1
Eunotia exigua	3	4	1	1	2	1	4	2	2	3	6.5	8.1
Chlamydomonas applanata var. *acidophila*	2	1	2		1		5		3	1	4.3	2.0
Hormidium rivulare	6	3	1		1		2	1	1	2	13.1	6.1
Zygogonium ericetorum	7	6			1			1		1	15.2	12.3
Dicranella sp. (protonema)	4	2	1		1	1		2			8.6	4.1
Characium sp.			1	1	1		2		1	1		
Lepocinclis ovum				2		1		1		2		
Chlamydomonas sp.		1				1	1	2				2.0
Dicranella sp. (adult)	2	1	1	2							4.3	2.0
Drepanocladus fluitans (adult)	3	3									6.5	6.1
Stichococcus bacillaris					1		1		1	1		
Nitzschia sp. type A					1		2					
Cryptomonas sp.							1	1				
Nitzschia ovalis									2			
Pinnularia microstauron									1	1		
Nitzschia palea							1			1		
Navicula sp.									1	1		
Drepanocladus fluitans (protonema)	1	1									2.1	2.0
Nitzschia sp. type B									1			
Navicula nivalis									1			
Ulothrix zonata										1		
Microthamnion strictissimum										1		
Typha latifolia	1	1									2.1	2.0
Juncus effusus	1	1									2.1	2.0

species to be considered as dominant it must be designated a score of 5. From these data it can be seen once again that Euglena mutabilis was considerably more abundant than any other species, occurring as abundant in 76% and 71% of the reaches for the two surveys. Pinnularia acoricola, Gloeochrysis turfosa, Zygogonium ericetorum and Hormidium rivulare were also considered as dominant species.

There were some differences in abundance between the two surveys, particularly with Nitzschia elliptica which was not only less common in late summer (Table 4.4) but also considerably less abundant.

Several species were not recorded abundant in any reach, but were reasonably commonly occurring; Lepocinclis ovum, Characium sp., Nitzschia subcapitellata and Chlamydomonas applanata var. acidophila are examples. The mosses Drepanocladus fluitans and Dicranella sp. were usually abundant at reaches where they occurred. Further details of abundance for individual species are given in Chapter 7.

4.5 Physical and chemical parameters by individual species

From the physical and chemical data collected for each reach, it was possible to determine the maximum, mean and minimum values for all parameters measured for each species (Table 4.9). Section I of this table also summarises the values collected for all the reaches in both surveys, giving the maximum, mean and minimum values. The data collected for the industrial effluent, site 15, were not included in the summary.

Summary of physical and chemical parameters

I		O.D. 420	conductivity (micro-mhos)	pH	acidity (as $CaCO_3$)	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4-P	NH_4-N	NO_3-N	SO_4-S	Cl	Si	O_2 %	temp. °C	current m s^{-1}
	max.	0.786	30000	3.0	64000	3550	52.8	2430	556	193	16.0	544	23000	3130	1.90	20.0	50.4	76	10.8	4.00	8600	255	114	106	21.2	0.46
	min.	0.004	600	1.5	110	3.90	0.05	6.60	4.00	0.09	0.006	0.35	7.52	3.72	0.001	0.007	0.05	0.01	0.05	0.15	250	14.0	4.5	10	5.9	<0.03
	mean	0.174	6450	2.7	7200	317	7.41	488	299	16.0	1.09	64.6	1111	258	0.24	2.16	4.2	1.92	2.20	0.57	2600	55.0	36.5	69.5	11.8	

species (II)		O.D. 420	conductivity (micro-mhos)	pH	acidity (as $CaCO_3$)	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4-P	NH_4-N	NO_3-N	SO_4-S	Cl	Si	O_2 %	temp. °C	current m s^{-1}
Euglena mutabilis	max.	0.786	30000	3.0	64000	3550	52.8	2430	556	193	16.0	544	23000	3130	1.90	20.0	50.4	76.0	10.8	4.00	8600	225	114	106	21.2	0.46
	min.	0.007	600	1.5	110	7.90	0.07	10.0	4.00	0.09	0.015	0.35	7.52	4.77	0.005	0.007	0.05	0.01	0.10	0.15	250	14.0	4.5	10	5.9	<0.03
	mean	0.189	5800	2.6	7300	371	7.45	433	269	10.0	2.05	96.9	1662	307	0.230	1.44	9.3	1.94	1.92	0.95	2600	65.5	41.5	68.5	11.35	
Pinnularia acoricola	max.	0.775	17500	3.0	64000	1780	52.8	2430	525	16.5	8.86	285	23000	2500	0.500	6.80	14.0	44.0	10.8	4.00	5900	255	114	106	21.2	0.41
	min.	0.014	2700	1.5	770	7.90	0.31	36.0	157	0.09	0.019	0.35	10.1	3.72	0.001	0.050	0.42	0.03	0.150	0.25	400	14.0	4.5	12	6.5	<0.03
	mean	0.025	7100	2.8	7400	396	7.00	538	347	8.80	1.03	71.5	1180	290	0.150	1.65	3.90	2.28	2.90	0.65	2600	65.8	39.6	74	14.2	
Gloeochrysis turfosa	max.	0.650	17500	3.0	15700	1250	52.8	2430	556	64.0	3.98	192	4900	1692	1.80	4.05	8.41	44.0	10.3	3.20	5500	173	97.0	97	21.2	0.41
	min.	0.001	600	1.8	850	3.90	0.42	25.2	60.1	0.54	0.006	6.30	21.0	3.72	0.001	0.008	0.05	0.01	0.05	0.22	250	14.0	4.5	12	5.9	<0.03
	mean	0.200	1200	2.7	8000	340	7.20	430	328	8.74	0.68	67.6	816	206	0.230	1.21	2.88	1.67	2.59	0.87	2300	47.4	34.1	76	15.2	
Nitzschia subcapitellata	max.	0.528	1000	3.0	15700	720	24.7	1120	556	37.6	2.32	113	2600	325	0.360	2.48	8.05	0.68	5.30	4.00	4500	255	62.0	97	21.2	0.20
	min.	0.005	400	2.5	770	13.6	1.35	26.0	138	0.09	0.007	0.35	12.5	3.72	0.003	0.050	0.05	0.04	0.31	0.26	400	14.0	6.0	12	7.1	<0.03
	mean	0.074	5100	2.7	4600	367	10.5	502	369	7.24	0.26	64.8	291	132	0.129	1.42	3.28	0.18	2.97	1.15	2000	71.5	35.9	70	13.5	
Nitzschia elliptica var. *alexandrina*	max.	0.490	7500	3.0	13900	785	16.5	905	525	54.5	2.10	113	2800	411	1.80	2.71	9.03	0.68	5.30	4.00	2900	255	170	100	15.6	0.25
	min.	0.005	1500	2.5	750	7.90	0.57	28.6	57.2	0.41	0.006	5.10	10.1	4.77	0.005	0.08	0.53	0.03	0.14	0.21	300	21.0	10.5	12	7.0	<0.03
	mean	0.092	5700	2.8	4400	329	8.90	394	361	11.6	0.75	69.1	353	131	0.284	1.53	3.73	0.17	2.80	0.52	1700	91.0	48.0	70	10.5	
Eunotia exigua	max	0.570	8000	3.0	10600	518	19.9	903	415	193	3.02	100	3250	212	1.75	2.15	8.05	1.80	10.5	2.90	4000	175	53.5	96	21.2	0.36
	min.	0.004	800	2.5	750	3.90	0.42	10.0	4.00	0.64	0.018	6.30	7.50	4.78	0.006	0.008	0.04	0.01	0.05	0.21	300	14.0	4.6	40	6.1	<0.03
	mean	0.047	4900	2.7	3500	161	5.98	228	238	22.6	0.75	43.0	407	86.2	0.405	0.710	2.31	0.51	1.84	0.56	1200	48.6	27.6	77	11.2	
Chlamydomonas applanata var. *acidophila*	max.	0.650	17000	3.0	15200	1250	29.0	1220	510	193	3.98	163	4800	1570	1.52	6.00	9.70	7.20	5.10	2.90	4900	155	97.0	95	20.1	0.36
	min.	0.004	1000	1.8	110	3.90	0.07	10.0	61.0	0.54	0.15	6.60	7.50	5.80	0.001	0.200	0.45	0.01	0.10	0.25	400	14.0	4.6	40	7.4	<0.03
	mean	0.150	6500	2.7	5400	334	7.15	434	275	24.1	1.10	74.6	973	270	0.310	1.75	3.30	2.00	1.75	1.48	1800	49.7	31.0	66	13.5	
Hormidium rivulare	max.	0.650	6500	3.0	7800	890	13.6	755	525	67.4	2.32	113	1020	411	1.90	2.62	6.21	0.43	5.10	3.20	3700	173	77.0	96	21.0	0.41
	min.	0.004	200	2.5	180	8.40	0.42	28.6	60.0	0.61	0.006	6.30	23.5	4.77	0.001	0.190	0.53	0.01	0.25	0.25	300	14.0	6.0	10	6.1	<0.03
	mean	0.080	3900	2.8	3200	291	6.30	298	287	18.2	0.38	50.8	229	109	0.395	0.950	2.43	0.15	2.28	0.47	1900	64.5	46.6	72	11.4	
Zygogonium ericetorum	max.	0.112	1000	3.0	15600	785	16.0	780	556	193	2.37	113	1310	411	0.950	2.48	6.50	0.88	3.90	2.50	5500	92.8	77.0	100	20.2	0.24
	min.	0.007	1300	2.5	750	8.10	1.40	26.0	67.0	0.09	0.23	0.35	12.5	10.4	0.010	0.050	0.05	0.04	0.10	0.26	400	14.0	4.5	30	7.1	<0.03
	mean	0.047	5300	2.8	5300	267	8.78	402	334	18.7	0.51	40.5	279	137	0.150	1.17	2.70	0.21	2.13	1.06	2200	39.4	37.8	61	11.7	
Dicranella sp. (protonema)	max.	0.150	5500	3.0	7800	385	21.2	870	520	64.0	1.24	113	390	411	1.75	2.62	6.21	0.88	5.30	2.90	2400	255	77.0	100	21.0	0.32
	min.	0.004	1500	2.5	850	3.90	1.89	30.8	61.1	0.71	0.018	31.0	23.5	3.72	0.003	0.180	0.53	0.04	0.51	0.26	400	18.0	10.5	55	7.1	<0.03
	mean	0.042	3600	2.8	2700	234	8.04	361	346	13.7	0.43	66.0	143	174	0.410	1.30	2.68	0.19	2.69	0.80	1400	80.9	29.9	75	11.7	
Characium sp.	max.	0.382	20000	3.0	20000	720	7.80	1510	520	57.0	8.86	285	4950	2500	1.52	5.82	11.3	76.0	5.20	2.90	3100	173	111	89	11.6	0.36
	min.	0.004	1500	1.5	900	3.90	0.31	6.60	61.1	1.14	0.007	6.60	34.5	5.80	0.013	0.020	0.48	0.24	0.05	0.38	400	33.7	7.6	30	7.6	<0.03
	mean	0.141	6500	2.6	6500	320	3.20	471	333	20.2	1.60	99.8	886	354	0.320	4.20	4.30	0.38	2.04	0.75	1800	101	50.0	61	10.8	
Lepocinclis ovum	max.	0.145	5000	2.8	8900	289	8.40	595	379	3.80	2.32	76.9	415	400	0.320	2.71	6.50	0.88	3.08	2.50	2400	29.5	77.0	100	9.1	<0.03
	min.	0.077	3000	2.5	4000	62.8	2.40	110	157	1.20	0.38	11.2	255	71.5	0.100	0.710	2.23	0.12	0.78	0.40	700	23.2	13.5	42	7.4	<0.03
	mean	0.131	4200	2.6	7900	187	4.80	426	326	1.85	1.26	44.2	340	218	0.220	1.69	5.24	0.35	1.53	1.32	1400	27.4	34.8	76	8.5	
Chlamydomonas sp.	max.	0.447	5500	2.9	7900	537	16.8	1120	520	1.40	1.24	113	415	411	0.340	2.62	5.54	0.57	2.60	0.70	1900	42.0	69.0	106	15.3	<0.03
	min.	0.005	3000	2.7	1900	45.3	7.8	595	221	0.77	0.22	28.9	101	55.0	0.020	0.550	3.05	0.04	0.78	0.38	1700	29.5	23.0	42	8.8	<0.03
	mean	0.159	4600	2.8	4400	109	12.05	725	390	1.17	0.77	64.2	217	180	0.180	1.34	4.00	0.26	1.47	0.55	1300	32.3	39.8	73	13.1	
Dicranella sp. (adult)	max.	0.187	4500	3.0	6200	330	15.5	551	556	4.64	2.86	50.0	610	176	0.23	2.84	4.52	1.60	5.30	0.60	5300	175	53.5	92	19.5	0.36
	min.	0.034	3000	2.6	1640	19.5	0.82	100	239	0.84	0.017	28.7	76.2	3.96	0.008	0.310	0.42	0.10	0.60	0.26	900	21.0	14.3	55	7.1	0.12
	mean	0.095	3700	2.8	2970	205	7.20	387	343	1.70	0.57	40.1	[illegible]	92.0	0.102	0.880	2.83	0.53	2.39	0.86	2100	55.0	35.0	77	14.3	
Drepanocladus fluitans	max.	0.041	4500	2.9	2000	330	7.60	210	325	1.50	0.69	50.0	110	38.5	0.500	0.800	1.91	0.43	5.30	0.50	1400	175	44.0	96	19.5	0.36
	min.	0.004	1700	2.6	1100	11.2	0.42	68	60.1	0.64	0.017	6.30	21.0	3.90	0.006	0.030	0.04	0.01	0.25	0.26	300	121	10.5	85	6.1	0.12
	mean	0.023	2700	2.7	1700	114	4.40	141	162	0.94	0.36	21.1	72.0	23.0	0.140	0.310	0.83	0.19	1.57	0.37	500	59.0	29.5	92	10.5	
Stichococcus bacillaris	max.	0.650	12500	2.8	10600	1250	9.25	905	520	7.40	2.80	151	345	890	0.500	2.62	4.64	1.05	2.60	0.50	1900	86.0	69.0	95	20.0	0.36
	min.	0.023	2000	1.8	1390	11.2	0.42	68.4	60.1	0.97	0.65	6.30	95.0	32.0	0.013	0.200	0.21	0.04	0.30	0.40	300	27.0	32.0	40	6.1	0.08
	mean	0.180	5500	2.4	3870	530	4.60	595	299	2.40	1.55	70.7	182	543	0.205	1.53	3.30	0.45	1.05	0.45	700	45.0	51.3	68	13.2	
Nitzschia sp. type A	max.	0.045	7000	3.0	3300	785	7.80	780	520	1.40	1.34	113	105	411	0.020	2.62	3.84	0.24	3.00	0.50	2400	42.0	69.0	90	21.0	0.05
	min.	0.041	4700	2.5	1800	385	5.00	284	348	0.85	0.18	50.0	70.0	4.86	0.001	0.800	0.63	0.04	2.60	0.50	1000	14.0	13.5	55	12.6	0.03
	mean	0.043	5700	2.8	2500	541	6.10	382	459	1.11	0.48	83.7	86.0	247	0.013	1.94	2.59	0.13	2.70		1800	25.7	49.7	70	16.3	
Cryptomonas sp.	max.	0.034	3500	2.9	1800	318	6.30	190	239	1.10	0.030	44.4	100	31.9	0.250	0.41	1.91	0.45	5.30	0.50	900	175	35.0	95	9.5	0.23
	min.	0.025	1800	2.6	1400	12.6	0.74	68.4	67.8	0.64	0.026	7.10	95.0	3.92	0.012	0.006	0.05	0.17	0.46	0.26	400	74.0	10.5	90	7.3	0.17
	mean	0.029	2600	2.7	1600	165	3.50	129	153	0.87	0.028	25.6	97.5	17.3	0.130	0.208	0.91	0.31	2.90	0.38	700	124	22.8	93	8.4	
Nitzschia ovalis	max.	0.150	5000	2.8	3800	385	21.2	870	377	0.85	0.14	50.1	155	81.3	0.008	1.23	1.68	0.10	2.60	0.50	2100	21.0	37.0	90	20.0	0.22
	min.	0.043	4700	2.5	1800	180	5.05	284	348	0.54	0.018	40.6	83.0	4.90	0.001	0.800	0.63	0.05	2.05	0.50	1000	40.0	13.0	88	9.5	0.03
	mean	0.096	4900	2.6	2800	283	13.1	377	362	0.69	0.079	45.3	119	42.9	0.004	1.05	1.16	0.07	2.32		1600	17.5	25.0	89	14.7	
Pinnularia microstauron	max.	0.043	4700	2.9	2100	385	6.50	284	348	0.85	0.030	50.1	105	4.86	0.100	0.800	2.52	0.10	4.00	0.50	1200	130	13.5	95	9.5	0.01
	min.	0.042	3000	2.5	1800	329	5.05	208	265	0.72	0.018	45.4	83.0	3.72	0.001	0.410	0.63	0.06	2.60	0.38	1000	14.0	11.0	90	7.3	0.03
	mean	0.042	3800	2.7	2000	357	5.75	246	306	0.78	0.24	47.8	94.0	4.29	0.060	0.610	1.58	0.08	3.30		1100	72.0	12.3	93	8.4	
Nitzschia palea	max.	0.006	2500	2.9	1900	119	7.60	152	148	1.50	0.48	10.6	36.0	38.5	0.080	0.230	0.38	0.14	0.55	0.50	600	24.6	44.0	96	11.4	<0.03
	min.	0.004	1800	2.7	1500	76.5	5.05	150	133	1.36	0.36	9.10	21.0	29.1	0.006	0.030	0.04	0.01	0.25	0.32	400	23.0	37.0	95	7.4	<0.03
	mean	0.005	2100	2.8	1700	97.8	6.30	151	140	1.43	0.42	9.80	28.5	33.5	0.043	0.130	0.21	0.08	0.40	0.41	500	23.8	40.3	95	9.4	
Navicula sp.	max.	0.043	4700	2.9	2100	385	6.50	284	348	0.85	0.030	50.1	105	4.86	0.100	0.800	2.52	0.10	4.00	0.50	1200	130	13.5	95	9.5	<0.03
	min.	0.042	3000	2.5	1800	329	5.05	208	265	0.72	0.018	45.4	83.0	3.72	0.001	0.410	0.62	0.06	2.60	0.38	1000	14.0	11.0	90	7.3	<0.03
	mean	0.042	3800	2.7	2000	357	5.75	246	306	0.78	0.24	47.8	94.0	4.29	0.050	0.610	1.58	0.08	3.30		1100	72.0	12.3	94	8.4	
Drepanocladus fluitans (protonema)	max.	0.028	2000	2.6	1500	12.3	0.26	77.0	64.2	1.14	0.69	6.60	110	32.2	0.800	0.210	1.30	0.43	0.64	0.50	400	86.0	39.0	95	11.3	0.36
	min.	0.023	2000	2.6	1400	11.2	0.57	68.3	60.1	0.97	0.65	6.30	95.0	31.0	0.013	0.200	0.48	0.26	0.30	0.40	300	27.8	32.5	85	6.1	0.31
	mean	0.025	2000	2.6	1400	11.7	0.59	72.5	62.1	1.05	0.87	6.50	103	31.6	0.410	0.205	0.89	0.35	0.47		400	56.9	35.5	90	8.7	
Nitzschia sp. type B	only occurrence	0.043	4700	2.5	1800	385	5.05	284	348	0.85	0.018	50.1	83.0	4.86	0.001	0.800	0.63	0.10	2.6	0.50	1000	14.0	13.5	90	21.0	<0.03
Navicula nivalis	only occurrence	0.061	4500	3.0	1600	40.0	1.89	230	383	4.54	0.54	89.0	390	190	0.015	2.15	3.30	0.31	1.05	0.50	3900	32.0	35.0	81	20.0	0.27
Ulothrix zonata	only occurrence	0.014	4500	3.0	3800	608	10.8	335	431	41.4	0.035	99.8	23.5	138	0.220	2.10	4.60	0.100	4.36	0.76	2300	173	59.0	92	8.2	<0.03
Microthamnion strictissimum	only occurrence	0.042	6500	2.9	5700	690	10.3	384	525	24.6	0.052	112	186	169	0.013	2.04	4.68	0.13	1.90	1.08	2700	88.0	57.0	37	11.6	<0.03
Typha latifolia	max.	0.043	4700	2.9	2100	385	6.5	284	348	0.85	0.018	50.0	105	4.82	0.100	0.80	2.32	0.10	4.0	0.5	1209	130	13.5	90	21.0	0.03
	min.	0.042	3000	2.5	1840	329	5.0	208	306	0.72	0.030	45.0	83.0	3.70	0.021	0.41	0.63	0.08	2.6	0.38	1028	14.0	11.0	97	7.8	0.03
	mean	0.042	3850	2.7	1970	357	5.8	246	265	0.78	0.015	47.5	94.5	4.25	0.060	0.61	1.58	0.08	3.3	0.38	1118	72.0	12.3	93	14.4	
Juncus effusus	max.	0.006	2500	2.9	1472	119	7.6	152	148	1.50	0.46	10.8	36.0	38.0	0.080	0.23	3.80	0.14	0.55	0.5	605	24.0	44.0	96	11.4	0.03
	min	0.004	1750	2.7	1900	76.0	5.0	150	133	1.36	0.36	9.1	21.0	29.0	0.096	0.03	0.04	0.01	0.25	0.32	429	23.0	37.0	95	7.4	[illegible]
	mean	0.005	2125	2.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

These data demonstrate the ability of the individual species to survive and in many cases, grow well, in very variable physical and chemical conditions. However, the very large range of concentrations of some elements, for example Fe 2300 - 7.5 mg l^{-1}, make it very difficult to determine whether an organism was inhibited by particular conditions. As already mentioned, the chemical parameters were in some instances so changeable from one season to the next, that it was impossible to determine whether more severe conditions, for instance in late summer, prevented a species from growing in a reach, or whether it was absent because of a lack of suitable inoculum. Therefore, whilst Table 4.9 provides some indication of the extremes the species can withstand, it does not necessarily determine what parameters influence the presence and absence of a species, with the exception of perhaps pH.

However, it was possible to say from the results that those species which occurred in 20% of all reaches and could tolerate pH values below pH 2.5, were also capable of growing in the presence of the largest concentrations of heavy metals recorded. Euglena mutabilis, Gloeochrysis turfosa, Chlamydomonas applanata and Characium sp. grew below pH 2.0, in acidity values in excess of 15000 mg l^{-1} $CaCO_3$, and at levels of Zn, 57.0 mg l^{-1}; Cu 3.9 mg l^{-1}; Co 4.0 mg l^{-1} and Ni 8.0 mg l^{-1}. Stichococcus bacillaris was recorded at pH 1.8, but not in waters containing the large concentrations of metals shown above. Several species were able to tolerate large concentrations of heavy metals but were not recorded at the extremely low pH values previously mentioned. For example, Hormidium rivulare, Zygogonium ericetorum and Dicranella sp. grew in water containing levels of metals in excess of: Zn, 67.4 mg l^{-1}; Cu, 2.32 mg l^{-1}; Co, 2.48 mg l^{-1} and Ni, 6.21 mg l^{-1}, but were not recorded below pH 2.5.

Where the occurrence of species was rare, then the values given for the chemical parameters will have little meaning, except to indicate the concentrations which the species were able to withstand.

4.51 The effect of pH and acidity on the distribution of species

As pH and acidity appeared to influence the occurrence of species to some extent (see 4.7), the distribution of the species over the pH and acidity ranges recorded, was examined in more detail. The number of reaches in which each species occurred, at a given pH value, are presented in Table 4/10. The maximum number of reaches per recorded pH value are also given.

The results demonstrate the increase in the number of species with increase in pH, up to pH 3.0. The number of species growing at and above pH 2.5 were greater than the number at pH values below this figure. However, this may be partly explained by the distribution of pH values recorded below pH 3.0. As mentioned previously, of the 95 reaches sampled in both surveys only 15 were below pH 2.5.

	pH 1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0
maximum no. of reaches possible at particular pH values	1	0	0	1	0	1	4	2	3	3	11	14	10	10	16	19
species																
Euglena mutabilis	1			1		1	4	2	3	3	8	12	10	8	12	17
Pinnularia acoricola	1			1		1	4	1	2	2	8	4	4	5	7	10
Gloeochrysis turfosa				1		1			2		5	7	7	6	7	8
Nitzschia subcapitellata											5	1	3	1	10	8
Nitzschia elliptica var. alexandrina											1	3			7	6
Eunotia exigua											2	5	4	2	8	2
Clamydomonas applanata var. acidophila				1			1	1	1		2	2		1	3	3
Hormidium rivulare											1	2	2	2	6	4
Zygogonium ericetorum											1	1	4	1	4	5
Dicranella sp. (protonema)											2		1	1	4	3
Characium sp.	1											1			2	3
Lepocinclis ovum											1	1	3	1		
Chlamydomonas sp.													1	2	1	1
Dicranella sp. (adult)												1	2		2	1
Drepanocladus fluitans (adult)												3	1		2	
Stichococcus bacillaris				1								2			1	
Nitzschia sp. type A											1				1	1
Cryptomonas sp.												1			1	
Nitzschia ovalis											1			1		
Pinnularia microstauron											1				1	
Nitzschia palea													1		1	
Navicula sp.											1				1	
Drepanocladus fluitans (protonema)												2				
Nitzschia sp. type B											1					
Navicula nivalis																1
Ulothrix zonata																1
Microthamnion strictissimum															1	
Typha latifolia											1				1	
Juncus effusus													1		1	

Euglena mutabilis, Pinnularia acoricola, Gloeochrysis turfosa, Chlamydomonas applanata, Characium sp. and Stichococcus bacillaris were the only species recorded below pH 2.5 and of these E. mutabilis, P. acoricola and Characium sp. were the most tolerant, growing at pH 1.5. However, all of these species were also commonly recorded at pH values above 2.5, with E. mutabilis being the most common species at all pH values recorded.

Microthamnion strictissimum, Ulothrix zonata and Navicula nivalis were only recorded at and above pH 2.9. M. strictissimum was commonly recorded in waters between pH 3.0 and 4.0, and pH 2.9 may be its lower limit for growth.

The distribution of species over the range of acidity values, expressed as Log_{10}, is shown in Table 4/11. The number of reaches at a particular acidity value are also given in the Table. The distribution of the acidity values and the occurrence of the species was more even than was found for the distribution over the pH range. The sudden increase in the number of species as recorded above pH 2.5, was not similarly demonstrated for any particular acidity value. There was, however, a gradual increase in the number of species and the number of occurrences of the species, as the acidity decreased in value.

All species, except Microthamnion strictissimum, were represented at least once, at an acidity value between 3.3 and 3.2 Log_{10} acidity and a value of around 4.3 log_{10} acidity was the upper limit for the majority of species present. At

$\log_{10}$ acidity	4.8	4.7	4.6	4.5	4.4	4.3	4.2	4.1	4.0	3.9	3.8	3.7	3.6	3.5	3.4	3.3	3.2	3.1	3.0	2.9	2.8	2.7	2.6	2.5	2.4	2.3	2.2
max. no. of reaches possible at particular acidity values	2	0	1	0	0	2	9	6	4	10	5	8	8	8	2	10	10	1	2	5	0	0	0	0	0	1	1
species																											
Euglena mutabilis	2		1			2	9	6	3	10	4	7	7	8	2	4	9	1	1	4						1	1
Pinnularia acoricola	2					2	3	2	2	7	3	6	5	5	1	7	2		1	1							
Gloeochrysis turfosa	1						3		2	4	4	3	4	5	1	5	8	1	1	2							
Nitzschia subcapitellata							1	1	1	2	1	3	5	2	1	8	2			1							
Nitzschia elliptica var. *alexandrina*								1		1	1	2	2	3		5	1		1								
Eunotia exigua								1	1		2		1			6	7	1	1	3							
Chlamydomonas applanata var. *acidophila*							4		1				1	1	1		1	1	1	2						1	1
Hormidium rivulare										1	1	2	1	4		3	2	1		1						1	
Zygogonium ericetorum							2			1		1	4	3	1	2				2							
Dicranella sp. (protonema)										1			1	1		4	2			2							
Characium sp.						1		1				2				1		1		1							
Lepoclinclis ovum										3		1	2														
Chlamydomonas sp.										1		1	1			2											
Dicranella sp. (adult)											1		1			3	1										
Drepanocladus fluitans (adult)																3	2	1									
Stichococcus bacillaris															1	1	1	1									
Nitzschia sp. type A														1		1	1										
Cryptomonas sp.																1	1										
Nitzschia ovalis													1				1										
Pinnularia microstauron																1	1										
Nitzschia palea																1	1										
Navicula sp.																1	1										
Drepanocladus fluitans (protonema)																	1	1									
Nitzschia sp. type B																											
Navicula nivalis																	1										
Ulothrix zonata														1													
Microthamnion strictissimum												1															
Typha latifolia																1	1										
Juncus effusus																1	1										

the highest acidity values of 4.8 - 4.3 $\log_{10}$ acidity, similar species occurred as were recorded at the lowest pH values; again, Euglena mutabilis grew at the highest levels recorded and was the most commonly occurring species for all recorded values.

4.6 Chemistry and flora of the industrial effluent

4.61 Chemistry

As mentioned in 2.2 the industrial effluent at site 15, was found to run at pH 3.0 only intermittently, therefore, the results were not included in 4.1 and are dealt with separately.

The heavy metal levels of the effluent were lower than in any of the mine drainages (maximum = Ni, C. 0.55 mg l^{-1}). The nutrient levels were generally high compared with mine waters and in particular the nitrate levels were high at all three reaches sampled.

4.62 Flora

During the first survey the only photosynthetic organism present at pH 3.0 was Euglena gracilis. Although this species was very abundant at several reaches on site 15, it was not recorded in any of the mine drainages. The increased pH on the second survey was accompanied by an increased and wide range of species. However, the flora did not include Euglena gracilis or any other acid mine water species.

4.7 Statistical analysis of data

In addition to the maximum, mean and minimum values given for the parameters measured in both surveys (see Table 4.9) Pearson correlation coefficient (r) was also computed for pairs of all the parameters measured, except current speed. The results are given in Table 4/12 for survey A and Table 4/13 for survey B. The (r) values given are only for correlations which were significant at and above the 95% confidence limit.

As can be seen from the data, there are several differences between the two surveys. For example, in survey A, there was a significant correlation between pH and the number of species at the 95% level, whereas in survey B there was no significant correlation at this level with pH, but there was a negative correlation between acidity and number of species. Other parameters that were correlated with the number of species at 95% limit in survey A were optical density ($r = -0.38$), conductivity ($r = -0.33$), Fe ($r = -0.310$) and SO_4-S ($r = -0.37$). In survey B, several other parameters were correlated at the same degree of significance, including acidity ($r = -0.33$), Al ($r = -0.29$), Ni ($r = -0.25$), PO_4-P ($r = -0.29$) NH_4-N ($r = 0.39$) and Cl ($r = 0.29$) as well as Fe and optical density. With the exception of Ni there was no correlation between the number of species and the concentration of any heavy metal ion.

Several ions appeared to be associated with one another, for example, Fe was significantly correlated with Al, SO_4-S, Co and Ni in both surveys. However, the majority of the major elements in solution were correlated with each other at one or both surveys and it is likely that they were not truly independent variables.

	Optical density	Conductivity	pH	Acidity	% oxygen	Temperature	Number species	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4 P	NH_4 N	NO_3 N	Cl	Si
Optical density																								
Conductivity	0.28																							
pH	-0.37	-0.61																						
Acidity	0.37	0.83	-0.47																					
% oxygen																								
Temperature	0.33																							
Number species	-0.34	-0.33	0.36																					
Na	0.48		-0.33																					
K																								
Mg	0.44	0.42	-0.48	0.38																				
Ca		0.54			-0.30	0.38				0.52														
Zn						-0.31																		
Cu	0.33	0.60	-0.61	0.48						0.36														
Mn	0.48	0.45	-0.47	0.35						0.79	0.46		0.48											
Fe		0.56	-0.47	0.35			-0.31					0.35	0.44											
Al	0.26	[illegible]	-0.57	0.79						0.37	0.31		0.83	0.45	0.41									
Pb											-0.34	0.55												
Co	0.38	0.74	-0.44	0.87									0.55	0.43	0.43	0.79								
Ni	0.34	0.76	-0.41	0.91									0.56	0.32	0.41	0.84		0.96						
PO_4 P		0.44	-0.33										0.61			0.48								
NH_4 N		0.47		0.44							0.36							0.44	0.39					
NO_3 N																								
SO_4 S	0.40	0.51	-0.39	0.45		0.51	-0.37			0.29	0.47			0.30	0.29	0.30	-0.30	0.51	0.45		0.33			
Cl	0.37	0.37		0.45						0.35			0.49	0.56		0.53		0.51	0.49					
Si	0.31	0.36	-0.29							0.51	0.48		0.59	0.68		0.53				0.45				

$r \geqslant 0.28$ at 95% significance level

$r \geqslant 0.36$ at 99% significance level

$r \geqslant 0.46$ at 99.9 % significance level

n = 46

	Optical density	Conductivity	pH	Acidity	% oxygen	Temperature	Number of species	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4 P	NH_4 N	NO_3 N	SO_4 S	Cl	Si
Optical density																									
Conductivity	0.52																								
pH	-0.36																								
Acidity	0.56																								
% oxygen																									
Temperature					-0.31																				
Number of species				-0.33																					
Na	0.36			0.60																					
K		0.33	0.54																						
Mg	0.54	0.62		0.44					0.58																
Ca																									
Zn																									
Cu	0.44	0.38	-0.49						-0.34																
Mn	0.52	0.42		0.39						0.71															
Fe	0.55		-0.43	0.45			-0.38	0.51		0.31			0.42	0.52											
Al	0.65		-0.44	0.63			-0.29	0.82					0.46	0.42	0.72										
Pb												0.37		0.37											
Co	0.55		-0.34	0.62				0.80		0.37			0.31	0.59	0.71	0.91									
Ni	0.73	0.34	-0.31	0.66			-0.25	0.77		0.47			0.39	0.56	0.63	0.92		0.92							
PO_4 P	0.70		-0.38	0.59			-0.29	0.72		0.31			0.47	0.46	0.58	0.89	0.35	0.84	0.86						
NH_4 N		0.29	0.29				0.39			0.30				0.47		0.32		0.32	0.32						
NO_3 N	0.35			0.39						0.29			0.30												
SO_4 S	0.61	0.37		0.48				0.55					0.36	0.46	0.62	0.74		0.73	0.73	0.69	0.31				
Cl					-0.32		0.29	0.40						0.35		0.51		0.35	0.33		0.70		0.38		
Si														0.30		0.33		0.41	0.35				0.42		

$r \geqslant 0.28$ at 95% significance level

$r \geqslant 0.36$ at 99% significance level

$r \geqslant 0.46$ at 99.9% significance level

$n = 46$

Conductivity is considered to be a measure of the total ionic concentration and was significantly correlated with a large number of ionic paramaters in the summer survey, but considerably fewer in the winter. For example, it was strongly negatively correlated with pH and positively correlated with acidity, Fe and Al in the late summer, but not so in the late winter.

As expected, many of the parameters measured varied inversely with pH, in particular those measured in summer when the pH values were generally lower. Although there was a significant positive correlation between acidity and the majority of the parameters which were correlated with pH in both surveys, pH and acidity were only significant in the summer period ($r \simeq -0.47$).

5. BRANDON PITHOUSE ACID STREAM

5.1 Water chemistry for the source of acid stream A

As mentioned in 2.22 monthly water samples were taken from the source of acid stream A from December 1972 until March 1975. The results of the analysis carried out for the 28 parameters measured, are given in Table 5.1, together with the maximum mean and minimum values. The standard deviation and standard error of the arithmetic means are also given.

The physical and chemical parameters measured were very consistent throughout the study. The total discharge from the source had a mean volume of 0.70 l s^{-1} S.E. + 0.086 and remained constant until month 24, when it increased considerably to 1.4 - 1.9 l s^{-1} and remained at this level for some time. The reason for this increase was considered to be due to an extended period of heavy rainfall, which caused a rise in the water from which the spring originated.

The parameters which were obviously affected by changes in total discharge were acidity, current speed, silicate, Co, Ni and Cl. Acidity and current speed increased with an increase in volume, whilst the other parameters mentioned above, decreased with increased volume.

Of the chemical parameters measured, pH was consistently the most stable, only varying from pH 2.60 to 2.70 S.E. $\pm$ 0.003. Other parameters that were also particulary constant at the source of the stream, included optical density, Na, K,

no.	date	$O.D._{420}$	conductivity micromhos	pH	acidity	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4-P	NH_4-N	NO_3-N	SO_4-S	Cl	Si	% O_2	temp	total discharge $l\ s^{-1}$	Eh mV	max. current speed $m\ s^{-1}$
1	10.12.72	0.038	1700	2.68	553	10.4	0.14	62.4	68.2	1.10	0.67	6.2	95.0	24.8	0.010	0.17	0.42	0.36	0.35	0.5	283	20.0	59.5	65	8.3	0.38		
2	10. 1.73	0.032	1650	2.65	652	11.0	0.20	57.6	60.5	1.05	0.70	6.2	111	27.2	0.001	0.21	0.45	0.37	0.48	0.5	323	18.8	55.5	60	9.5	0.38		
3	10. 2.73	0.024	1650	2.62	688	11.5	0.15	58.0	57.7	1.12	0.71	6.0	102	26.5	0.010	0.20	0.50	0.35	0.38	0.5	313	22.5	48.5	74	8.8	0.35		
4	13. 3.73	0.020	1750	2.65	748	11.2	0.13	66.0	60.5	1.13	0.77	6.3	120	28.8	0.012	0.20	0.54	0.38	0.37	0.5	311	17.5	44.5	68	8.2	0.33		
5	12. 4.73	0.012	1800	2.65	744	11.3	0.30	62.4	58.0	1.02	0.83	6.3	108	29.8	0.009	0.22	0.50	0.38	0.37	0.5	460	22.5	42.0	65	7.9	0.34		
6	11. 5.73	0.018	1950	2.60	620	11.1	0.18	58.0	53.4	1.00	0.79	6.3	102	28.5	0.008	0.15	0.41	0.43	0.35	0.5	308	21.5	36.0	73	9.5	0.32		
7	14. 6.73	0.013	1900	2.65	618	11.3	0.08	64.5	67.5	1.14	0.68	5.5	94.0	28.8	0.013	0.18	0.48	0.36	0.28	0.5	107	22.0	33.5	84	9.0	1.00		
8	25. 7.73	0.014	2000	2.65	1390	12.3	0.06	68.3	64.2	1.08	0.69	6.6	110	31.2	0.017	0.16	0.43	0.43	0.54	0.5	321	20.2	39.0	85	9.9	1.15		
9	24. 8.73	0.014	2000	2.65	700	11.3	0.10	75.0	67.2	1.24	0.75	7.1	116	34.6	0.003	0.11	0.47	0.54	0.58	0.65	546	14.7	37.0	66	9.8	1.12		
10	20. 9.73	0.016	2000	2.65	785	11.4	0.10	73.9	64.3	1.32	0.83	7.6	126	36.7	0.013	0.12	0.46	0.51	0.62	0.79	451	27.4	38.2	68	8.9	1.00		
11	18.10.73	0.021	1950	2.65	843	10.3	0.03	67.0	67.0	1.23	0.85	7.6	144	36.5	0.018	0.25	0.57	0.56	0.41	0.81	378	25.4	38.4	70	8.4	0.80		
12	16.11.73	0.031	1850	2.65	1280	11.3	0.12	64.8	65.2	1.30	0.78	7.2	105	34.2	0.050	0.21	0.43	0.62	0.19	1.06	305	29.5	40.1	70	4.5	0.82		
13	12.12.73	0.016	1800	2.65	985	11.4	0.48	58.6	62.1	1.31	0.69	7.2	98.4	32.3	0.051	0.24	0.51	0.24	0.88	0.81	361	24.6	56.8	65	4.7	0.61		
14	15. 1.74	0.012	1450	2.65	825	11.7	0.82	52.5	53.0	1.23	0.57	6.2	82.0	25.0	0.014	0.20	0.52	0.10	2.30	0.75	468	25.4	61.5	63	5.5	0.52		
15	18. 2.74	0.023	2000	2.65	1300	11.2	0.43	77.0	60.0	0.97	0.65	5.3	95.0	32.0	0.060	0.23	0.41	0.26	0.40	0.45	312	27.8	32.5	70	4.4	0.38		
16	12. 3.74	0.018	1850	2.65	1000	11.1	0.33	60.5	66.3	1.08	0.73	6.8	103	33.2	0.003	0.21	0.46	0.33	0.42	0.53	410	26.0	36.6	79	7.8	0.36		
17	22. 4.74	0.024	1600	2.60	850	11.9	0.92	54.3	61.0	1.06	0.59	6.1	100	23.0	0.020	0.23	0.41	0.63	0.38	0.63	415	23.0	52.0	74	8.1	0.38		
18	20. 5.74	0.019	1800	2.65	792	14.6	0.11	78.0	100	1.58	1.00	8.9	140	43.2	0.010	0.65	0.27	0.20	0.65	-	554	33.8	38.5	80	8.4	0.41		0.35
19	17. 6.74	0.031	1900	2.65	800	11.4	0.23	76.8	81.2	1.34	0.68	6.6	115	36.2	0.003	0.32	0.50	0.41	0.40	0.52	601	25.3	30.5	77	8.8	0.37		0.28
20	28. 7.74	0.023	1750	2.65	772	11.3	0.10	72.0	74.0	1.24	0.82	7.0	116	33.8	0.003	0.47	0.23	0.19	0.72	0.42	638		32.4	81	8.7	0.36		0.29
21	13. 8.74	0.031	1800	2.65	790	11.2	0.15	73.0	72.6	1.23	0.84	7.2	122	34.4	0.006	0.25	0.52	0.18	0.82	0.65	554	24.0	43.5	87	9.9	0.37		0.29
22	12. 9.74	0.041	2000	2.65	1200	11.1	0.24	66.1	66.5	1.21	0.86	7.6	129	37.9	0.008	0.16	0.41	0.25	0.28	0.47	853	32.1	40.5	76	9.1	0.36		0.27
23	14.10.74	0.017	2000	2.65	1000	12.1	0.26	78.0	81.0	1.21	0.82	7.1	125	35.1	0.009	0.18	0.44	0.31	0.14	0.60	633	33.0	41.0	82	8.7	0.37	+610	0.28
24	16.11.74	0.020	1350	2.70	1200	12.2	0.36	68.0	70.0	1.01	0.66	5.4	95.0	26.5	0.009	0.17	0.42	0.29	0.41	0.45	330	26.6	40.0	84	9.0	0.91	+570	0.30
25	17.12.74	0.025	700	2.65	1100	11.3	1.02	66.1	62.4	1.23	0.83	6.9	129	51.8	0.017	0.24	0.52	0.42	0.35	0.35	357	37.2	39.0	80	7.9	1.46	+570	0.44
26	18. 1.75	0.024	1750	2.65	1300	11.6	0.14	71.2	66.2	1.29	0.87	7.8	134	38.3	0.007	0.24	0.58	0.32	0.65	0.70	575	25.0	36.0	78	7.6	1.68	+590	0.58
27	15. 2.75	0.017	1800	2.65	1400	10.5	0.73	62.0	58.6	1.05	0.69	6.5	100	28.1	0.002	0.04	0.04	0.10	0.80	0.25	500	21.0	33.0	80	7.4	1.90	+570	0.60
28	14. 3.75	0.067	1750	2.65	1350	10.7	0.24	60.0	56.5	1.13	0.75	6.9	109	30.4	0.018	0.24	0.54	0.14	0.15	0.25	495	20.2	33.5	82	6.4	1.40	+580	0.45
maximum		0.067	2000	2.70	1400	14.6	1.02	78.0	100	1.58	1.00	8.9	144	51.8	0.060	0.65	0.58	0.63	2.30	1.06	853	33.1	61.5	87	9.9	1.90	610	0.60
mean		0.024	1767	2.65	1197	11.4	0.29	66.1	65.9	1.18	0.75	6.7	111	32.4	0.014	0.22	0.45	0.35	0.54	0.56	434	24.3	41.4	74	8.1	0.70	581	0.38
minimum		0.012	1350	2.60	553	10.3	0.03	52.5	53.0	0.97	0.57	5.2	82.0	23.0	0.001	0.04	0.04	0.10	0.14	0.25	107	14.7	30.5	60	4.4	0.32	570	0.28
standard deviation		0.0112	261	0.018	168	0.78	0.268	7.39	9.63	0.129	0.095	0.79	15.3	6.08	0.015	0.11	0.132	0.144	0.390	0.175	150	4.34	10.66	7.74	1.58	0.454	4.32	0.117
standard error		0.004	96.7	0.003	62.2	0.15	0.051	1.39	1.83	0.025	0.018	0.15	2.90	1.15	0.003	0.02	0.025	0.027	0.075	0.033	28.5	0.821	1.94	1.46	0.29	0.086	0.91	0.022

Zn, Cu, Pb and to a lesser extent Eh, oxygen and Mn concentrations. All other parameters measured, but not mentioned so far, fluctuated to some extent.

The overall chemical nature of the water from the source of Brandon Pithouse Acid Stream A, was typical of acid mine drainage water as found in the two surveys (see Table 4.1). However, it was not considered to be a particularly severe chemical environment compared with other acid streams, in that hydrogen ion concentration, acidity and heavy metal content of the water, were lower than the values recorded at some sites.

The nutrient content was also lower than the mean values obtained from the survey of acid streams, although they were not considered to be sufficiently low as to limit growth of the photosynthetic organisms growing in the stream.

5.11 Statistical analysis

Apart from the mean, standard deviation and standard error of the monthly results, Pearson's correlation coefficients were also determined for each pair of parameters and a correlation matrix formed as given in Table 5.2. Only values which were significant at 95%, 99% and 99.9% confidence levels are given.

As can be seen from the results, many of the cations and some anions were significantly correlated with each other at values above the 95% limit; this indicated that they were not totally independent of each other.

	Optical density	Conductivity	pH	Acidity	% oxygen	Temperature	Total discharge	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4 P	NH_4 N	NO_3 N	SO_4 S	Cl	Si
Optical density																									
Conductivity																									
pH																									
Acidity																									
% oxygen																									
Temperature				-0.57																					
Total discharge				0.38																					
Na																									
K		0.46				0.38																			
Mg									-0.42																
Ca					0.39			0.64		0.71															
Zn								0.49		0.47	0.67														
Cu									-0.42	0.51	0.51	0.56													
Mn										0.38	0.58	0.81	0.79												
Fe										0.57	0.54	0.57	0.85	0.78											
Al		0.69								0.57	0.47	0.63	0.75	0.67	0.75										
Pb						-0.79																			
Co								0.61			0.68	0.60	0.46	0.46	0.38	0.39									
Ni	-0.40				-0.33																				
PO_4 P																									
NH_4 N																									
NO_3 N					-0.38							0.44					0.39		0.55						
SO_4 S				0.40						0.39	0.38	0.43	0.44	0.58	0.49	0.38									
Cl				0.40							0.52	0.49	0.43	0.43	0.52			0.46							
Si					-0.58					-0.58			-0.38				-0.43								

$r \geqslant 0.37$ at 95% significance level

$r \geqslant 0.47$ at 99% significance level

$r \geqslant 0.59$ at 99.9% significance level

n= 28

As mentioned in 5.1, several parameters related to the total discharge, in particular, acidity. These observations were supported by the statistical analysis, with acidity being significantly correlated with total discharge at the 95% level, and Co, Ni and Si negatively correlated at 90% significance. As can be seen from the data there was no significant correlation between pH and acidity, or between these parameters and others, such as Fe and SO_4, which are often associated with them.

5.2 Sampling programme

Owing to the changes which occurred in the stream below the confluence at reach 8 (see 3.5) the results have been separated into three parts. The first deals with those data collected from October 1972 to July 1974; the second from July to November 1974, and the third from November 1974 to March 1975. The number of samples taken in each period is summarized in Table 2.2.

Although reaches 7 and 7b represent the sampling reaches on stream B (see Fig.3.2), for convenience they have been included with the other reaches on stream A. The results presented in Fig. 5.1 to 5.27 do not include water chemistry results taken when the streams were in flood, as these were not considered to be representative of the conditions which normally prevailed in the stream.

5.3 Water chemistry data for reaches 1 to 19, from October 1972 to July 1974

5.31 Overall trends in physical and chemical parameters

The 26 physical and chemical parameters recorded for the 19 10 m sampling reaches are presented in Fig. 5.1 - 5/2 7. There was a general decrease in the mean concentrations of many of the chemical parameters measured, with an increase in pH values, as the water flowed from source to the confluence with Redburn, at reach 16 (Fig. 3.2). This decrease in ionic concentration was demonstrated most markedly by acidity, Fe, Al, Mn, Si, SO_4-S and heavy metal concentration. Other parameters such as current speed, temperature, dissolved oxygen, Cl and NH_4-N tended to remain stable, whilst the remaining parameters either fluctuated eg. Ca, Mg, or increased eg. NO_3-N, as the pH increased down the stream.

5.32 Stream A, reach 1-6

With the exception of flooding due to surface run-off and the occasions when the reservoir was frozen, the volume of water flowing down stream A was relatively constant throughout the period of study. From reaches 1 – 4, upstream of the reservoir, the stream was never known to dry up, even under extreme climatic conditions. Likewise, the physical and chemical parameters for reaches 1 to 6 were very stable and where there were large variations in the reaches downstream of the source, this could be accounted for by surface run-off from the tip.

The majority of parameters decreased downstream of reach 1, although the pH, Eh and Na concentrations, remained the same over this section. The mean values of acidity, PO_4-P, Fe and Al showed quite a large decrease downstream of

Fig. 5.1

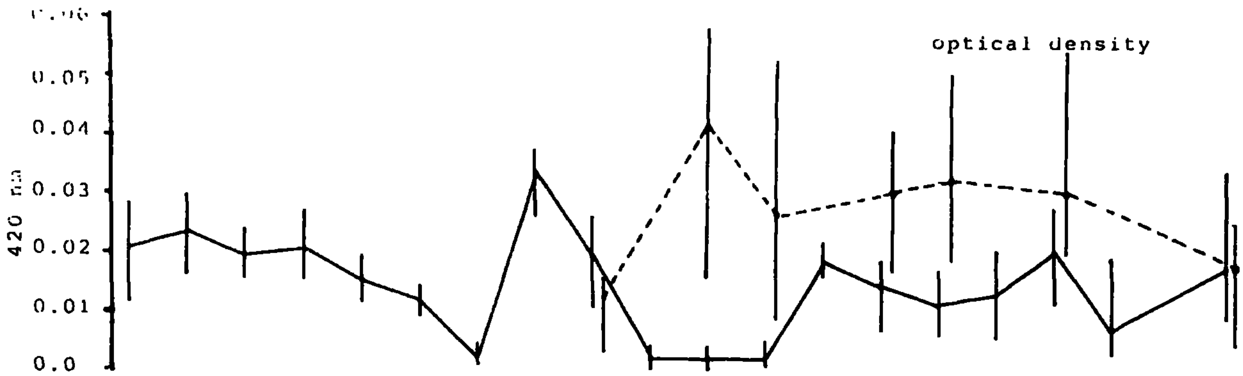


Fig. 5.2

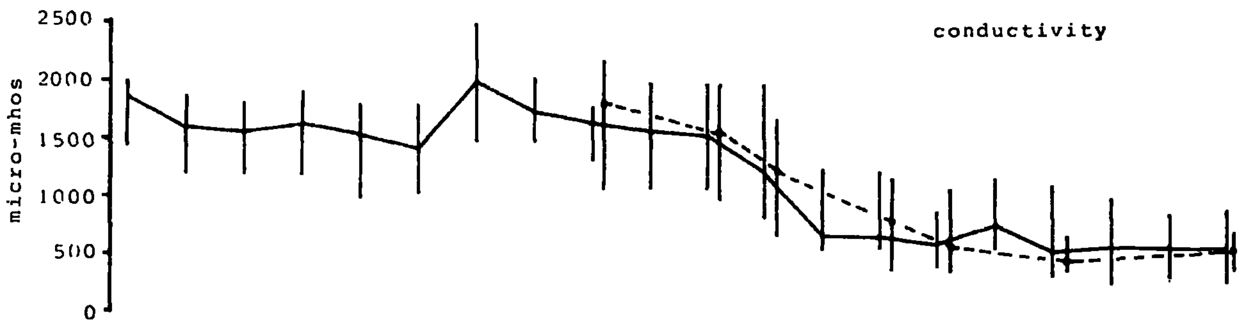


Fig. 5.3

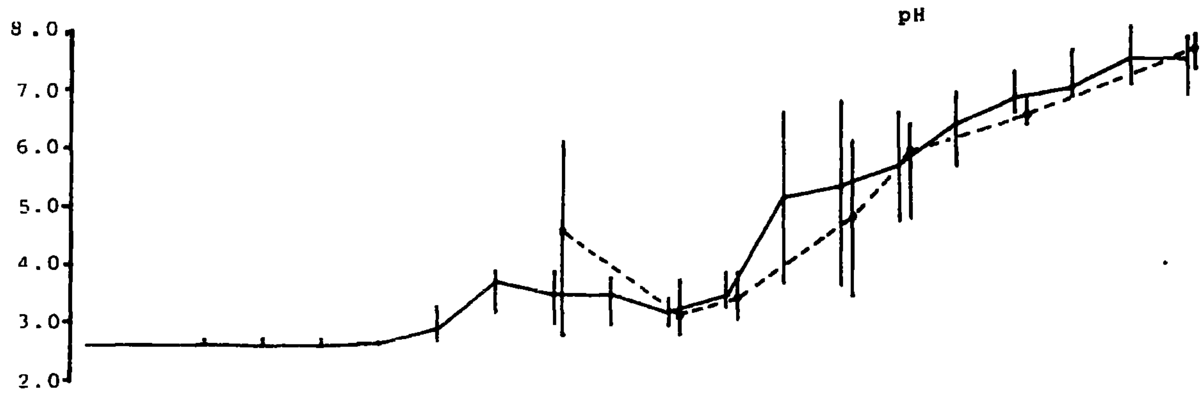


Fig. 5.4

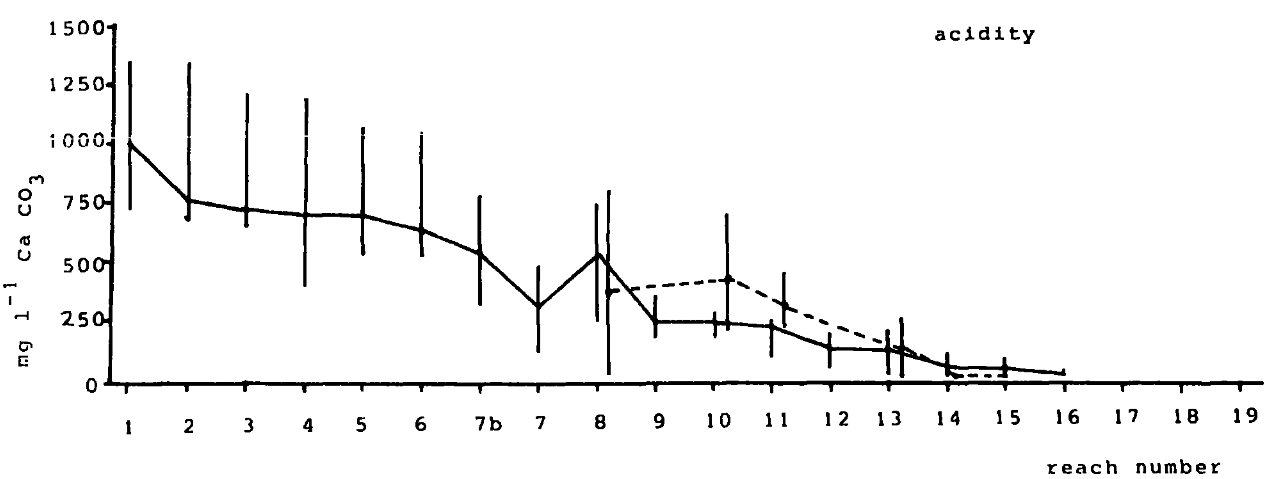


Fig. 5.5

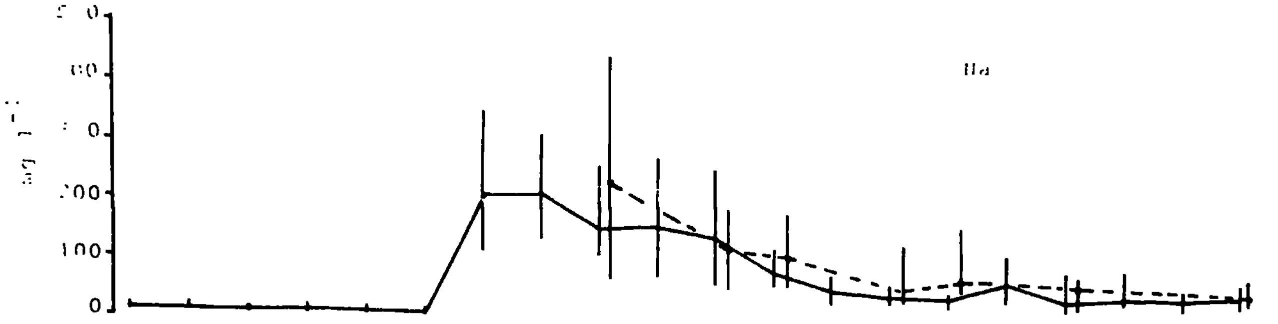


Fig. 5.6

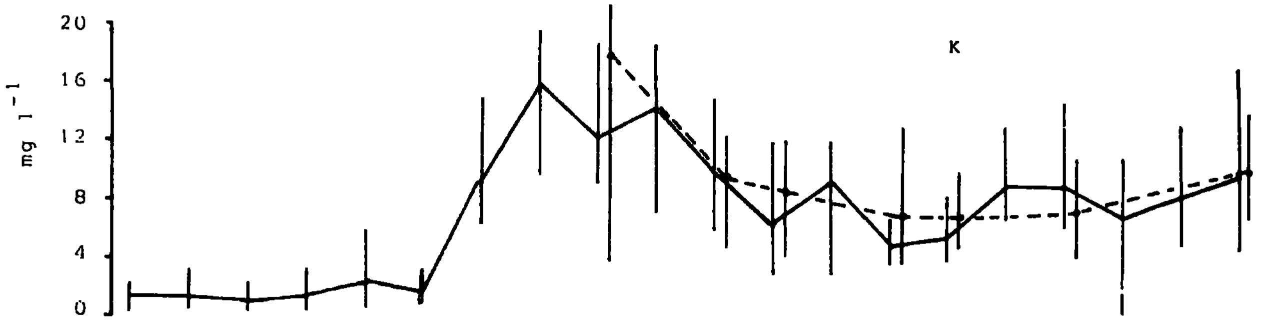


Fig. 5.7

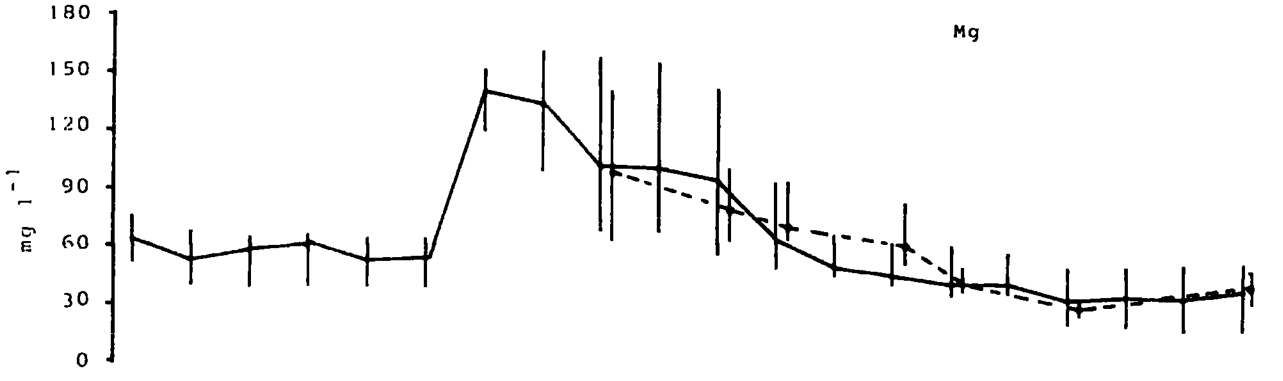


Fig. 5.8

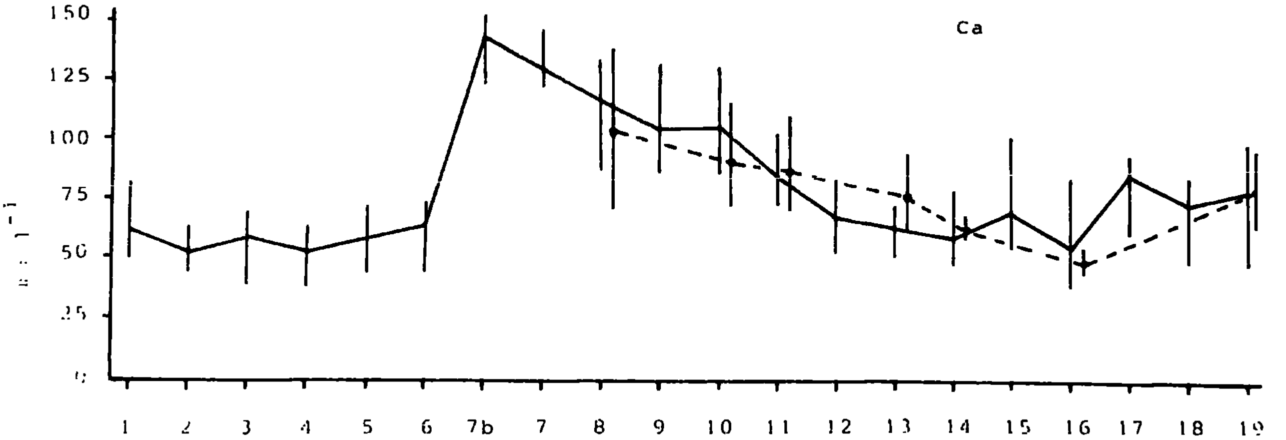


Fig. 5.9

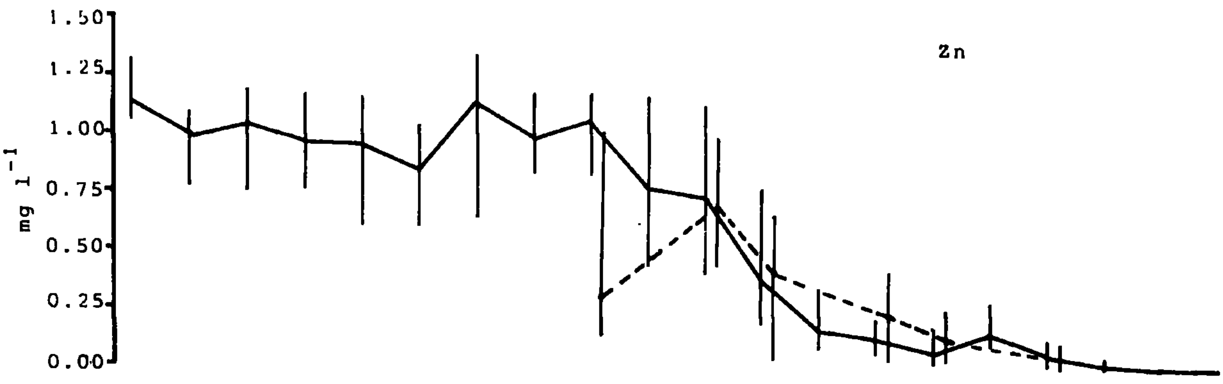


Fig. 5/10

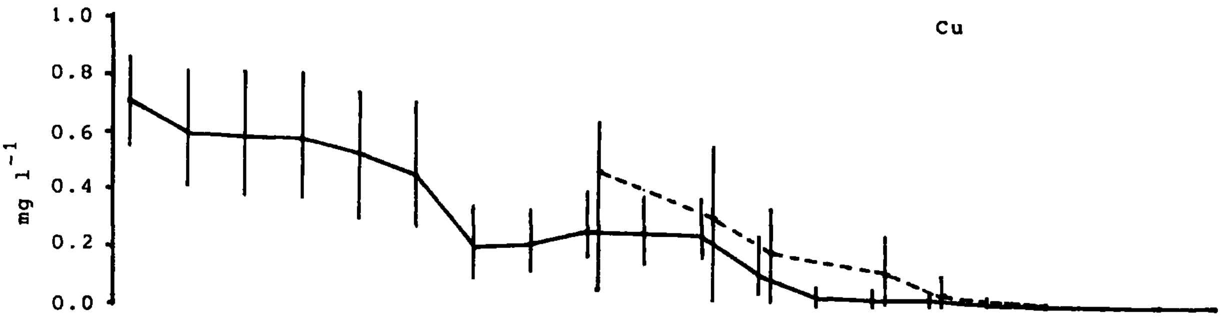


Fig. 5/11

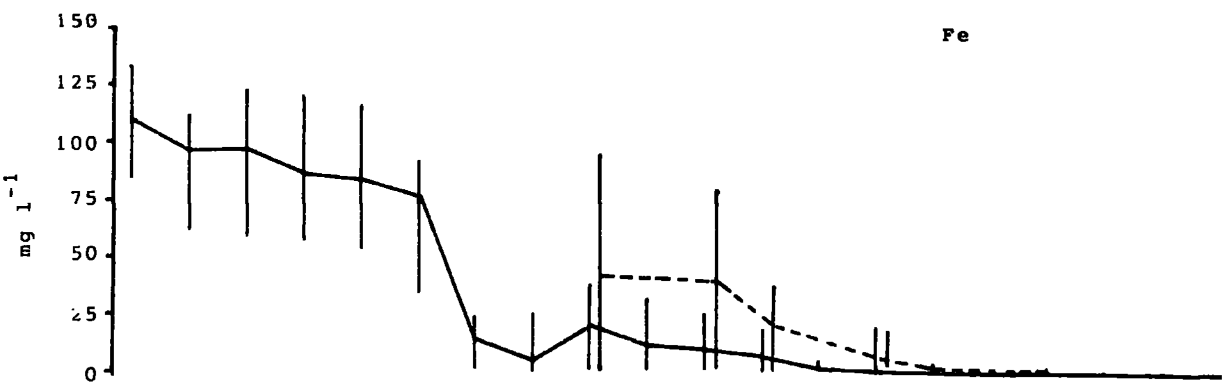


Fig. 5/12

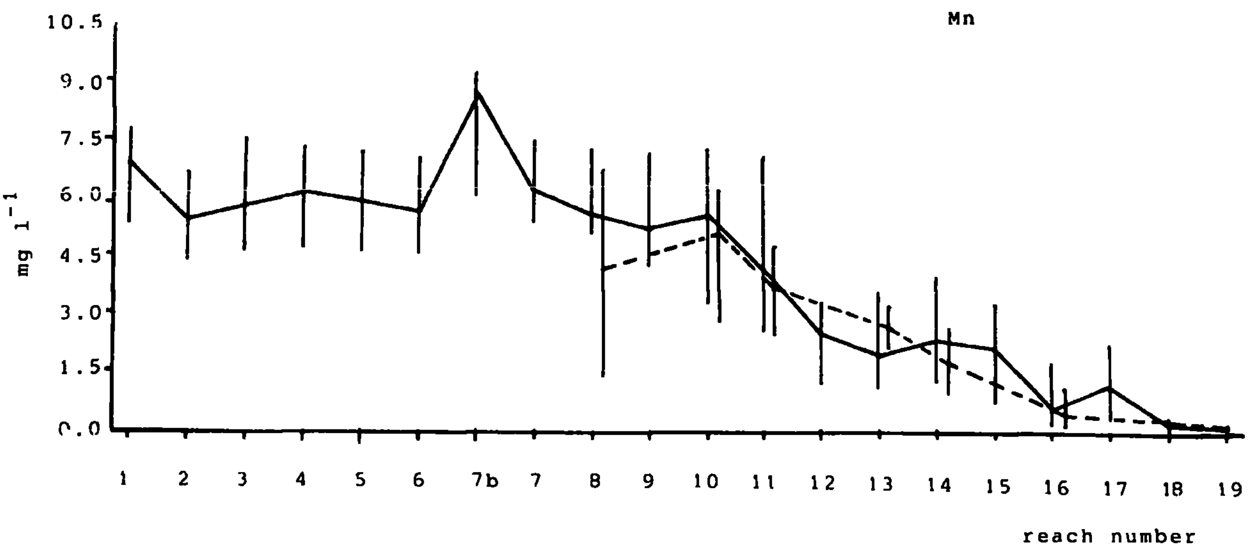


Fig. 5/13

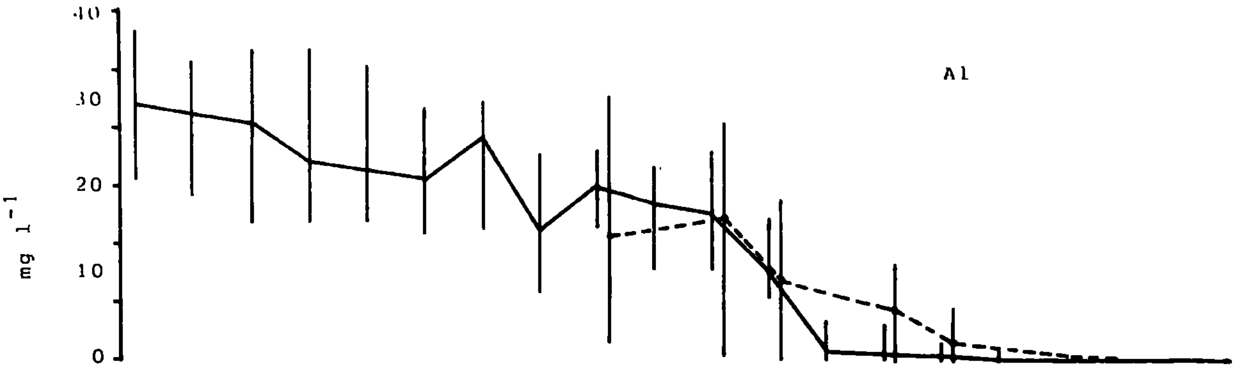


Fig. 5/14

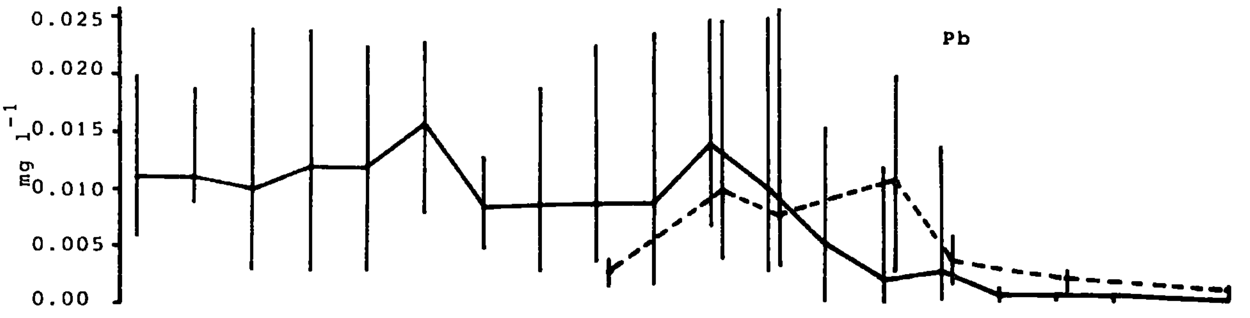


Fig. 5/15

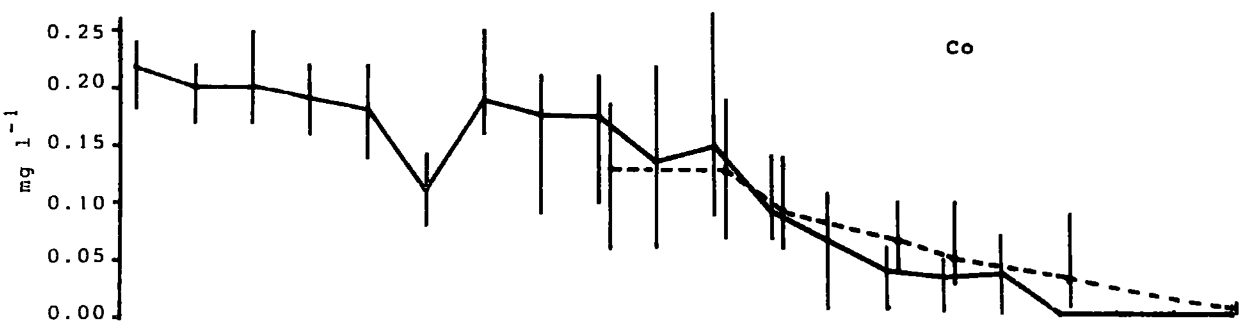


Fig. 5/16

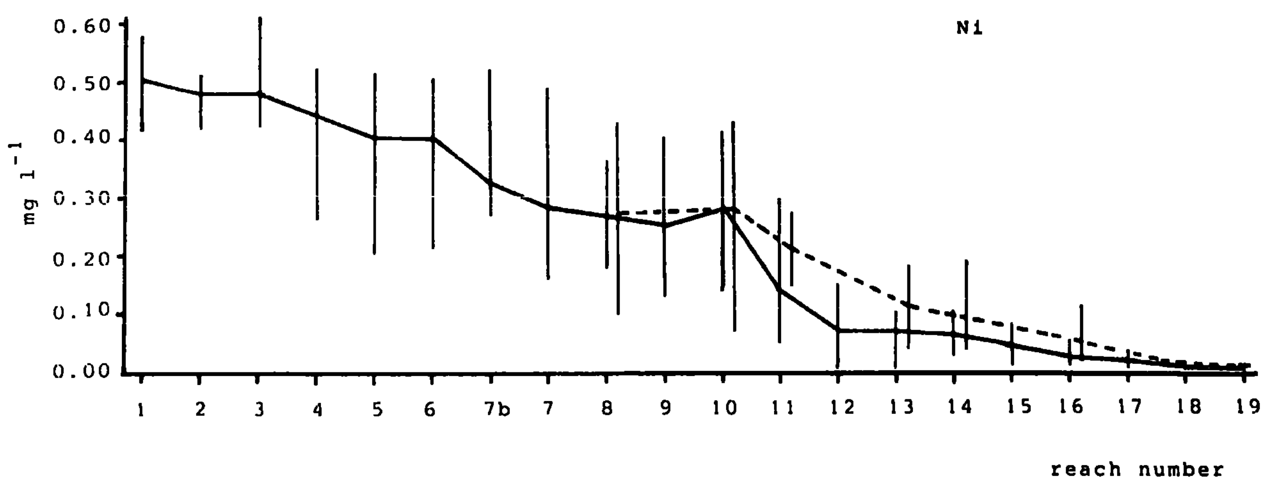


Fig. 5/17

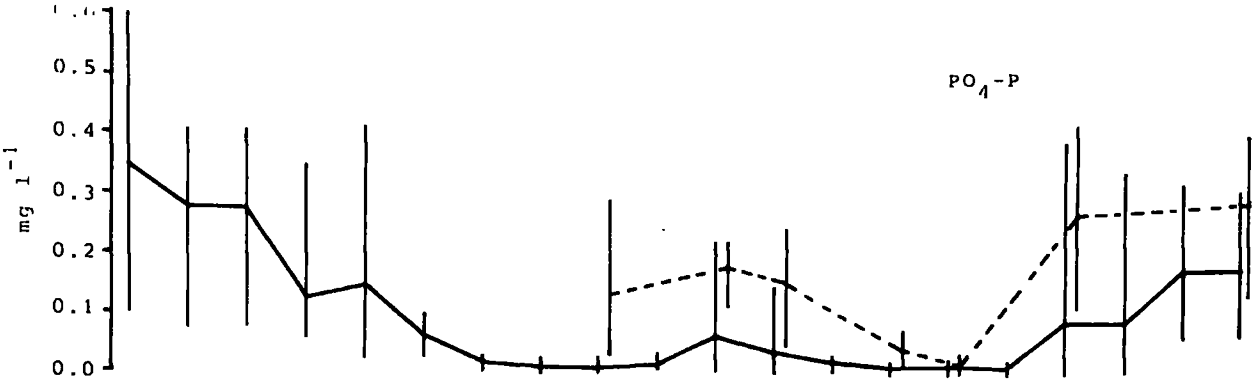


Fig. 5/18

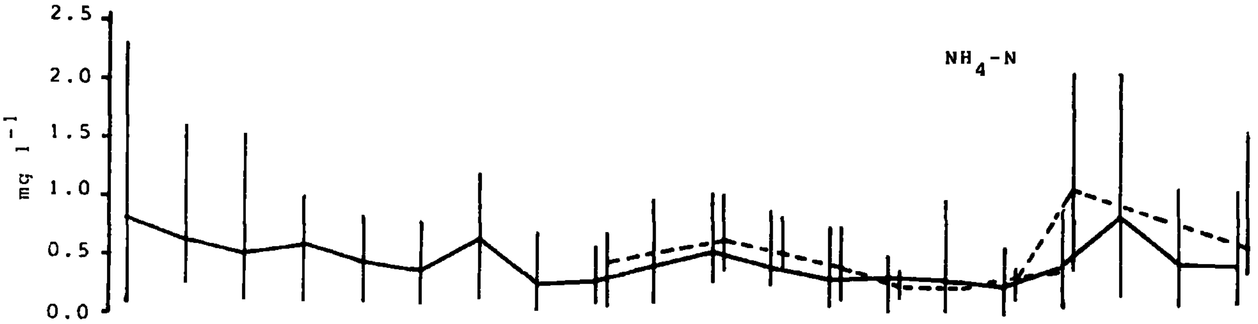


Fig. 5/19

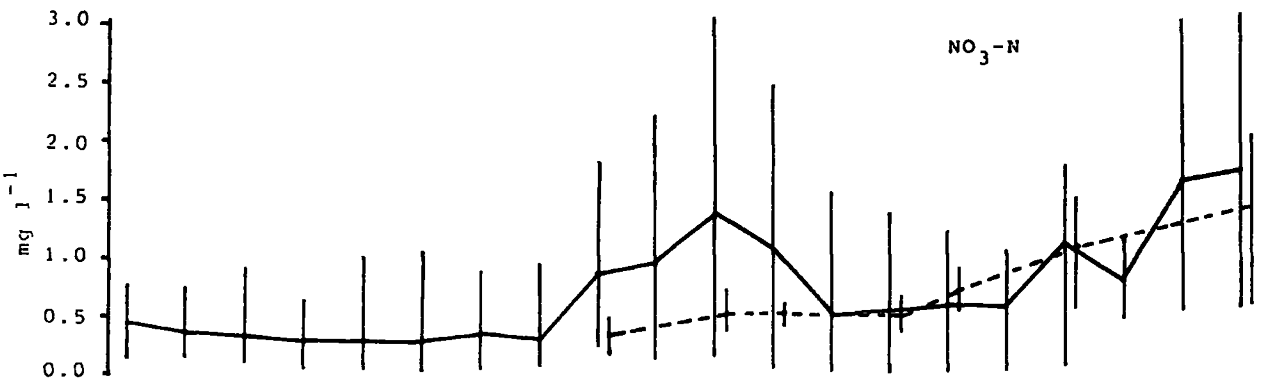


Fig. 5/20

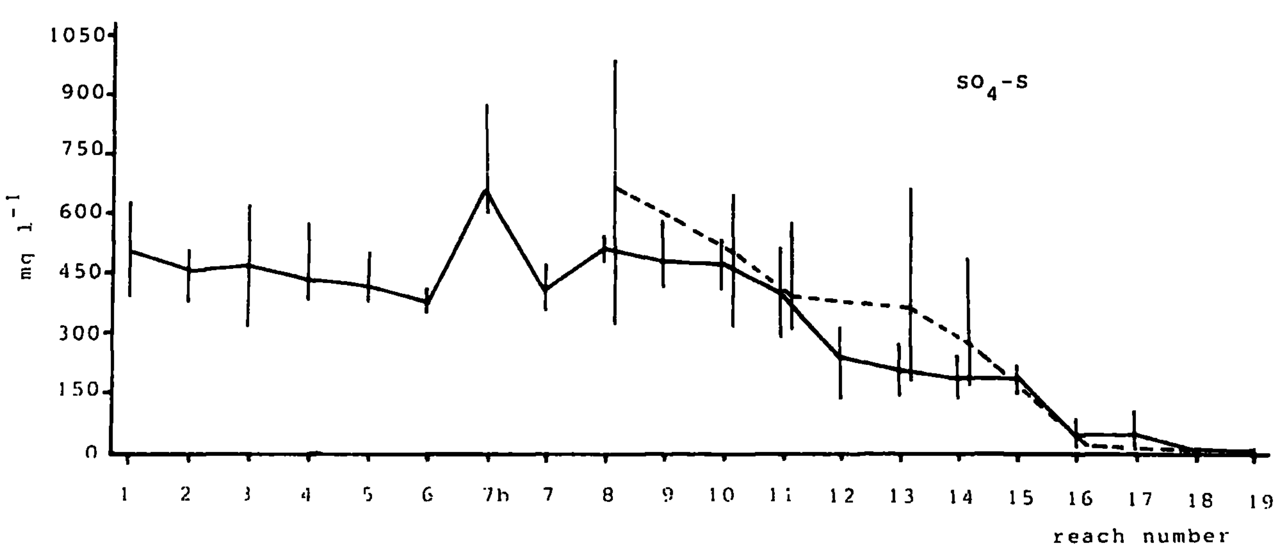


Fig. 5/21

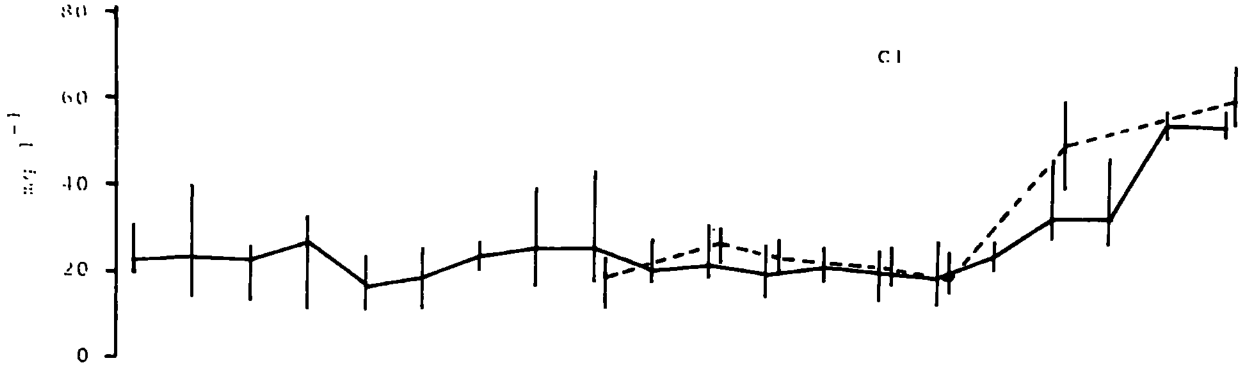


Fig. 5/22

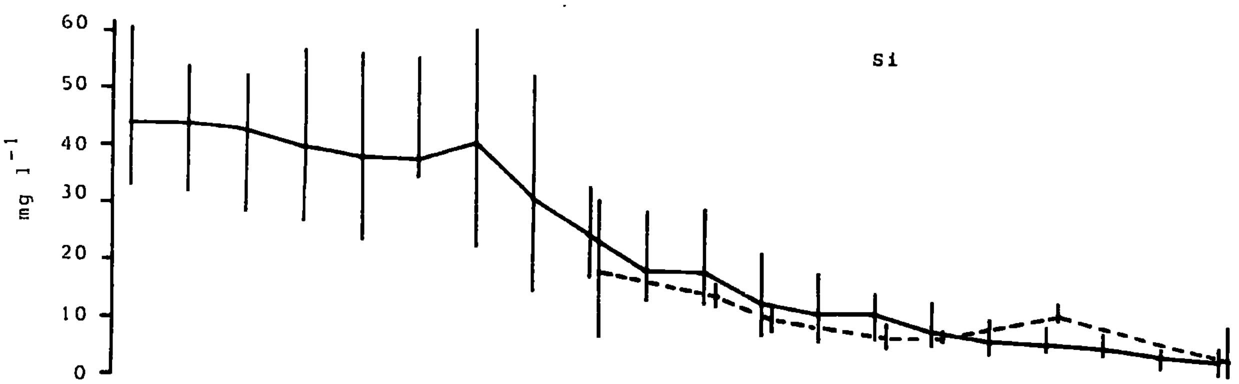


Fig. 5/23

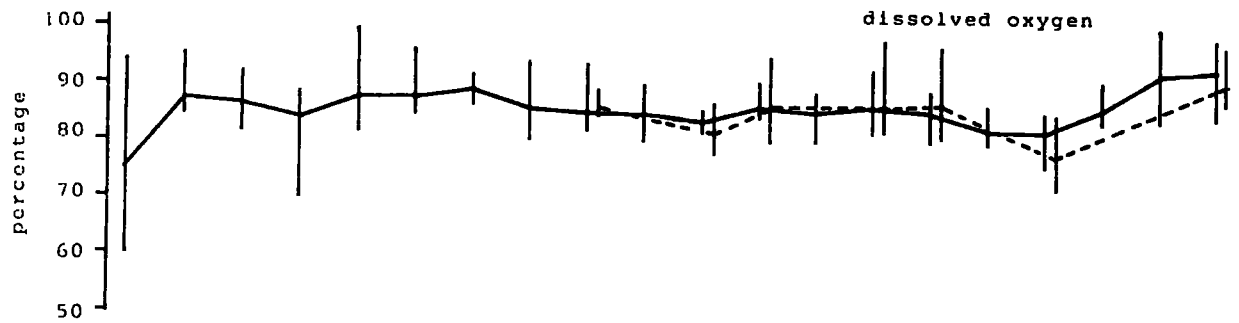


Fig. 5/24

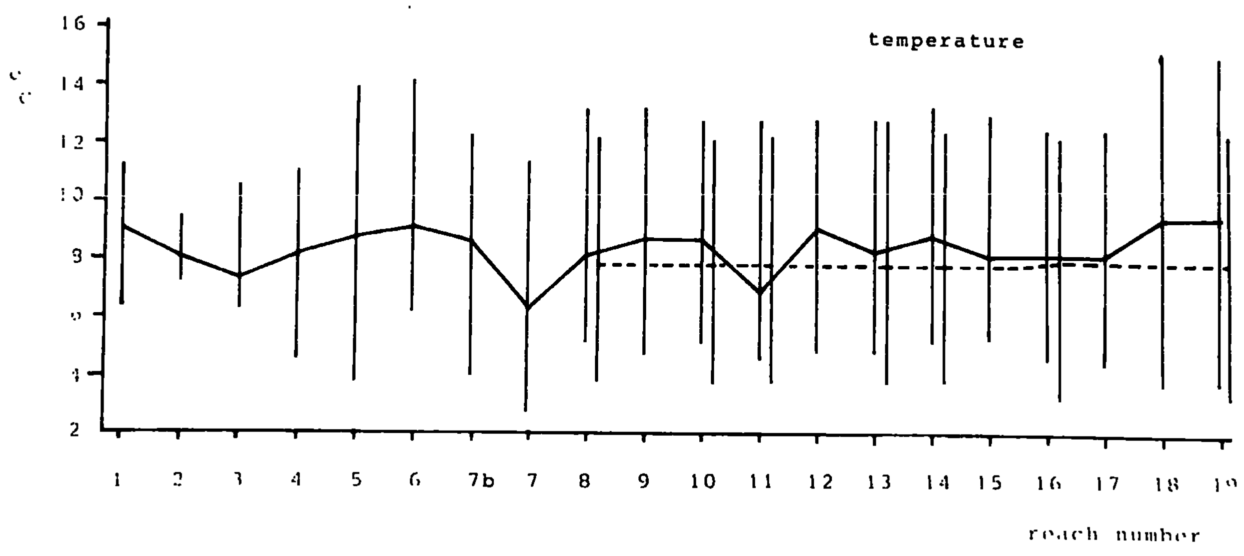


Fig. 5/25

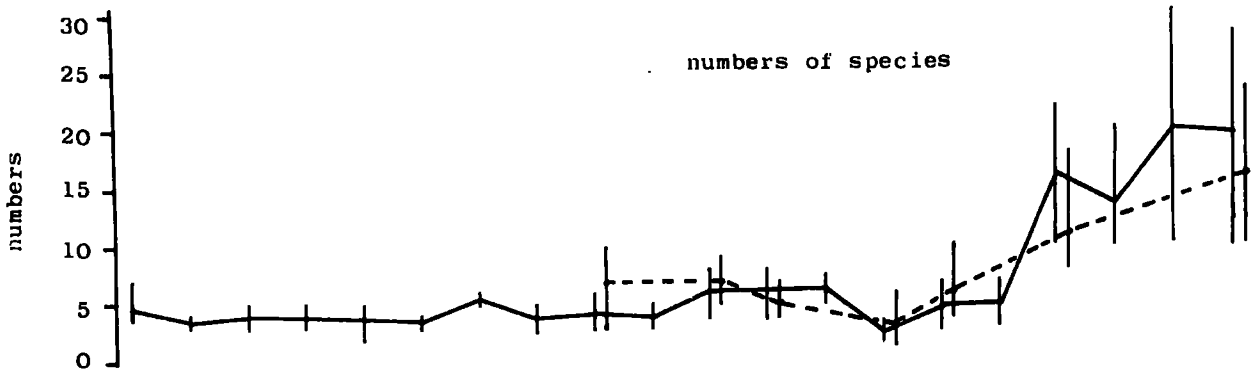


Fig. 5/26

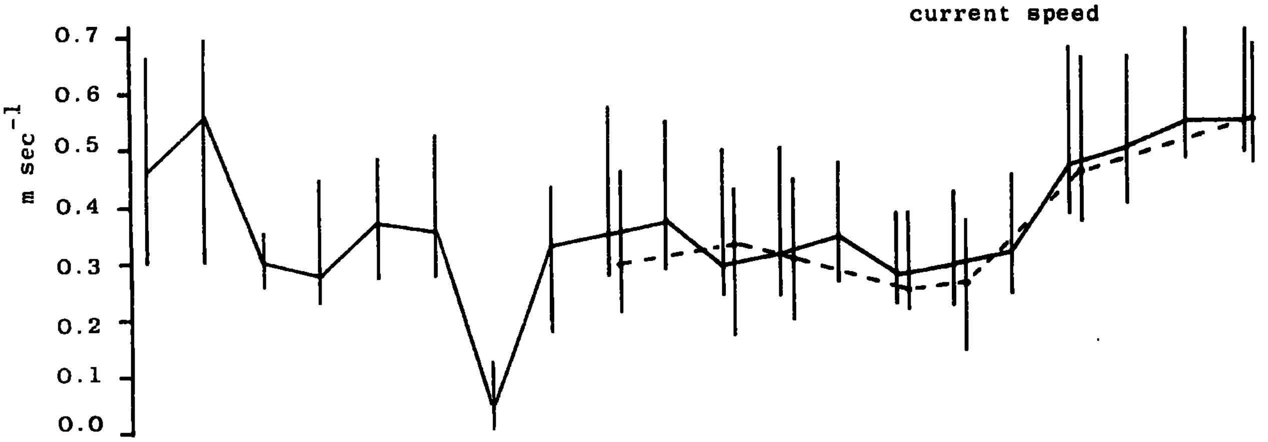
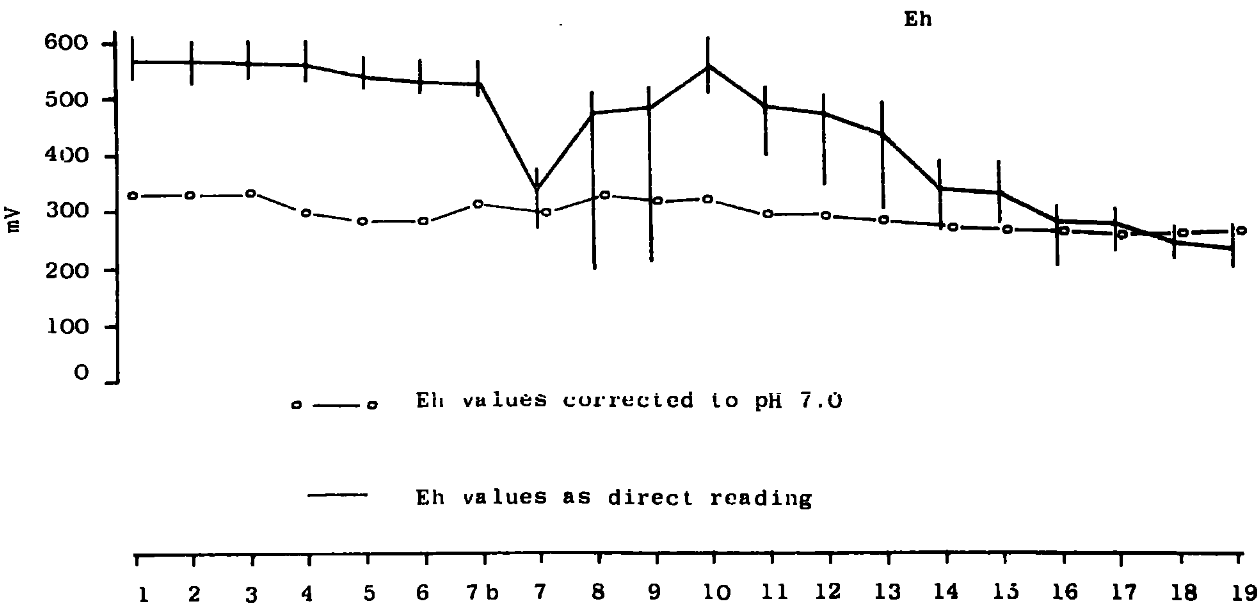


Fig. 5/27



reach 1, whereas dissolved oxygen content increased from a mean value of 75% to 87% between reaches 1 and 2 (Fig. 5/23).

The holding reservoir (reaches 4 and 5) did not have any marked effect on the chemistry of the water, although several chemical values did alter slightly. Those parameters that decreased slightly included levels of Cu, Mn, Co, Ni, Cl and NH_4-N, whilst levels of Ca, K, PO_4-P and O_2 increased.

5.33 Stream B, reaches 7 and 7b

A comparison of the chemistry of stream B, reach 7b, with stream A, reaches 1-6, shows that whilst the levels of acidity, some heavy metals and anions were similar, there were several major chemical differences between the two waters. The pH, Na, K, Ca, Mg, Mn and SO_4-S values were consistently greater than the values for stream A, whilst Fe and Cu were considerably lower.

The nutrient status of both streams was similar, except the PO_4-P concentration, which was less in stream B (mean 0.02 cf. 0.1 mg l^{-1} PO_4-P). (Fig. 5/17). At stream B, reach 7 the pH increased to a mean value of pH 3.8 (Fig. 5.3) as a result of the addition of non acidic mine water from the tip. As mentioned in 4.32 this resulted in the precipitation of Fe and Al from solution. In addition to the consequential decrease of those elements from the water, several other constituents also decreased as a result of both dilution and possibly precipitation. However their decrease was only slight compared with that of Fe and Al. With the exception of Ni, the levels of heavy metals and nutrients in the water at reach 7 remained reasonably constant. The decrease in temperature (Fig. 5/24) observed at this reach, was due to the water being piped underground for several hundred metres and also the addition of surface run-off below reach 7b.

5.34 Downstream confluence of stream A and B at reach 8

The result of the two streams meeting was to produce a water of mean pH 3.5 at reach 8 (Fig. 5.3). The increase in pH of stream A from 2.6 caused a large amount of iron oxide to be precipitared out on the stream bed and an associated drop in Fe concentrations from 75 mg l^{-1} to 20 mg l^{-1} (Fig. 5/11).

The overall effect on the water chemistry, was to produce a water that consisted of elements in concentrations proportional to the levels found in the two streams, at reaches 6 and 7. Where one stream contained a greater concentration of a particular element, the level in the mixed water tended to be nearer that value, for example, acidity, Ca and Mg. The only parameter that increased significantly above the mean concentration in both streams was NO_3-N (Fig. 5/19). The reasons for this increase were not clear but it was thought to have been due to agricultural run-off.

5.35 Reaches 9 - 11

There was a general decrease in the value of all parameters measured between reach 9 and reach 11, except for the mean values of pH, acidity, O.D. and oxygen, which remained about the same. The mean concentration of several ions decreased more markedly than others over this section; these included levels of K, Al and the heavy metals, except Pb, all of which decreased by almost half the value at reach 9.

At reach 10, a small acidic seepage entered the stream (see 3.43) and caused an increase in the concentration of many ions. In particular, the levels of Pb, Co, Ni and some nutrients, increased above the values at reaches 8 and 9. However, by reach 11 the levels of those and other elements had decreased again to below the levels at reach 8. This decrease was due to the diffusion of the water through an extensive bed of Drepanocladus fluitans and the influence of a small volume of non acidic drainage water.

5.36 Eshwood to Redburn, reaches 11 - 16

A general decrease in the levels of chemical parameters was maintained over this section of the stream. Between reach 11 and 12 two small alkaline tributaries entered the acid stream (see 3.43), resulting in a sharp increase in pH from 3.5 to 5.2. Consequently downstream of the confluence of these tributaries further precipitation of iron took place, (Fig. 5/11) and a flocculent precipitate was produced. Although, dilution was responsible for the majority of the decreases in concentrations which occurred, some ions decreased more than others and may have been incorporated in an Fe-organic complex. In particular, levels of Al, Mn, Cu and NO_3-N were found to decrease significantly at this point.

The presence of the fine flocculent material caused a considerable increase in the optical density values. Between reaches 11 and 16, where the stream enters Redburn, the mean concentration of the heavy metals decreased markedly, for example, Zn, 0.4 to 0.05 mg l^{-1}; Cu 0.11 to 0.009 mg l^{-1}; Co and Ni 0.15 to 0.02 mg l^{-1}. Other ions which have not been mentioned but which also decreased were acidity, Eh, Mg and SO_4-S.

5.37 Redburn to the River Deerness, reaches 16-19

The effect of Brandon Pithouse Acid Stream on Redburn was dependent on the volume of water in both streams. With the flow down the acid stream being reasonably constant, the greatest effect on Redburn was shown during the summer months, when the water level in Redburn was low. During these periods the pH of Redburn immediately below the confluence was depressed to below pH 7.0 and the levels of heavy metals increased. However, when the flows in Redburn were average and above, the effects on the water chemistry of the stream were reduced and at times difficult to detect.

As can be seen from a comparison of the water chemistry at reaches 18 and 19, the Redburn has an insignificant effect on the River Deerness.

5.4 Water chemistries for samples, July - November 1974

As mentioned in 3.5, during this period the pipe feeding the reservoir on stream A was broken and consequently, no water flowed out of the reservoir, thus leaving reaches 5 and 6 dry. As a result of the lack of water from stream A. the total flow of the stream below reach 8 was from stream B.

5.41 Reach 1 - 6

The only chemical changes which occurred as a result of

Table 5.3

Comparison of the mean physical and chemical parameters for reach 8, Brandon Pithouse acid stream during the periods October 1972 to March 1975. All concentrations are as mg l^{-1}.

	OD 420 nm	conductivity micro mhos	pH	acidity as $CaCO_3$	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4-P	NH_4-N	NO_3-N	SO_4-S	Cl	Si	% O_2	temperature °C	current speed ms^{-1}	Eh Mv
Mean value October 1972 to July 1974	0.002	1680	3.5	530	153	12.5	105	118	1.15	0.27	5.46	24.0	21.2	0.009	0.18	0.27	0.02	0.33	0.88	508	29.3	25.4	85	8.0	0.35	
Mean value July to November 1974	0.004	2000	6.3	18.0	400	32.2	138	138	0.10	0.34	3.5	1.4	2.8	0.002	0.07	0.12	0.05	0.40	0.44	950	17.0	7.8	86	10.3	0.30	+200
Mean value November 1974 to March 1975	0.016	1450	2.85	750	62.0	4.6	68.3	71.4	1.10	0.62	6.4	92.0	28.4	0.003	0.18	0.40	0.16	0.50	0.26	356	25.4	31.0	86	5.6	0.36	+550

the altered flow regime, were downstream of the reservoir at reaches 5 and 6. Although the level in the reservoir was considerably reduced there was sufficient water entering the reservoir to permit normal sampling at reach 4. As a result of the work being carried out on the tip (see 3.32), additional alkaline water was diverted into stream B, below reach 7b, this caused an increase in pH at reach 7 from 3.8 to 6.2. Apart from the changes mentioned above, the water chemistry of these reaches remained the same as reported in the previous section (see 5.33 and 5.34).

5.42 Reach 8

The effects of the changes brought about by the cessation of stream A at reach 6, during this period were most obvious at reach 8. The result of the changes are given in Table 5.3.

Apart from an increase in the mean pH, from 3.5 to pH values ranging between pH 4.5 and 6.2, many chemical parameters were considerably altered, compared with the pre-July 1974 period (Table 5.3). For example, the acidity decreased from a mean of 500 mg l^{-1} to 16 mg l^{-1} $CaCO_3$; Zn, 1.04 mg l^{-1} to 0.10 mg l^{-1}; Fe, 250 mg l^{-1} to 0.90 mg l^{-1}; Al, 21.2 to 2.3 mg l^{-1}. In contrast, concentrations of Na, K, Mg, Ca, SO_4-S, PO_4-P and O.D. increased significantly during this period.

In addition to changes in the chemical parameters at reach 8, there were also accompanying physical changes. Because most of the Fe which normally precipitated out over this stretch of the stream (see 5.3) originated from stream A, there was a marked reduction in the amount of iron precipitate during this period. Although some iron continued to be deposited over this area, by November 1974 much of the substrata were covered with silt rather than iron oxide.

5.43 Reaches 9 - 19

The same changes in water chemistry, as described above for reach 8, were observed at reaches downstream of this point. However, the effects of the increased pH were reduced at reaches 10 and 11 due to the presence of acid seepage, and many of the parameters remained much the same as in the pre-July period. The amount of precipitation over this section of the stream was also reduced considerably, although not quite to the same extent as was observed at reach 8.

5.5 <u>Water chemistries for samples November 1974 - March 1975</u>

5.51 Introduction

As explained in 3.53, the pipe feeding the reservoir was repaired at the end of November 1974 and the acid water started once again to flow its full length, as was the situation before July 1974. The immediate result was to produce water at reach 8, which was chemically similar to that flowing at this reach before July 1974. However, due to a more efficient flow through the new pipe into the reservoir, the volume of water mixing with stream B, at reach 8, increased. In addition, the volume of water from stream B decreased and consequently further changes in the water chemistry occurred at reach 8.

5.52 Reach 8

The pH of the water at reach 8 decreased from pH 6.2

(the value in November) to 2.85 in January 1975, this value was also lower than that recorded in the pre-July 1974 period (see Table 5.3). Many of the changes recorded when the pH increased from pH 3.5 to 6.2 (see 5.42) had reversed by March 1975. In addition to a decrease in pH, levels of Na, K, Mg, Ca and SO_4-S also decreased because of the lower levels present in stream A water. The concentrations of Cu, Pb, NO_3-N, NH_4-N, O_2, temperature and current speed remained similar to previous values recorded at that reach.

The remaining parameters all increased when compared with the July to November 1974 period, and a few also increased above the values of the pre-July period. Those that were higher than at any time previously recorded included Zn, Cu, Mn, Fe, Al, Ni, PO_4-P. Because the pH was less than 3.0 during the November to March 1975 period, the increased level of Fe did not lead to an increase in the amount of Fe precipitated out, although some of it was deposited on the stream bed.

5.53 Reaches 9 - 19

The general increase in concentration of many of the parameters and the decrease in pH described for reach 8 in 5.52, were also found at reaches downstream of the confluence. Once again the changes were least at reach 10, although even here there was a tendency to follow the general trend shown at other reaches.

The main effect of the decrease in pH was demonstrated at reach 13, where the non acid tributaries entered the stream. Here the pH increased above pH 3.5 and because only a small amount of the increased Fe in solution had precipitated out at reach 8, the majority of the iron still remained in solution until reach 13, where it precipitated out as a result of the addition of alkaline water. The section of the stream from reach 13 to 15, was affected by both the increase in iron hydroxide precipitate and by a lower pH and increased heavy metal content.

The effect of the acid stream on Redburn was greater by March 1975 than at any time previously recorded. During this period the acid stream water entering Redburn was at pH 4.9, compared with a previous mean value of pH 6.6 and there were also greater levels of Fe and Al in solution. Although the pH of Redburn at reach 16 remained at pH 7.0, the bed of the stream was covered with a layer of Fe and Al oxides. The concentrations of heavy metals at reach 16 were also considerably greater, for example, the concentration of Zn increased from 0.04 to 0.15 mg l^{-1}.

The effect of the increased loading on Redburn was not reflected in the chemistry of the R. Deerness downstream of the confluence, presumably, tributaries to Redburn were sufficiently large as to dilute the acid water by the time it reached the R. Deerness.

5.54 Statistical analysis

In addition to indicating the range of values recorded at each reach, for all the parameters measured (see Fig. 5.1 - 5/27), Pearson's correlation coefficients were also determined for all pairs of parameters measured. The (r) values for those r ≥ 0.24 at 99% significance level

r ≥ 0.34 at 99.9 % significance level

n= 136

	Optical density	Conductivity	pH	Acidity	% oxygen	Temperature	Total discharge	Number species	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4 P	NH_4 N	NO_3 N	SO_4 S	Cl	Si
Optical density																										
Conductivity																										
pH		-0.77																								
Acidity		0.66	-0.82																							
% oxygen																										
Temperature																										
Total discharge																										
Number species		-0.47	0.71	-0.52		0.30																				
Na	-0.24	-0.39		-0.24																						
K									0.60																	
Mg		0.71	-0.43					-0.28	0.75	0.35																
Ca		0.47							0.80	0.35	0.87															
Zn		0.78	-0.78	0.88				-0.56			0.49															
Cu		0.65	-0.77	0.88				-0.49					0.87													
Mn		0.83	-0.89	0.84				-0.61			0.60	0.28	0.93	0.78												
Fe		0.55	-0.71	0.89				-0.44	-0.41			-0.31	0.79	0.79	0.71											
Al		0.75	-0.86	0.92				-0.54			0.45		0.96	0.89	0.95	0.84										
Pb			-0.33	0.29									0.29		0.28											
Co		0.73	-0.81	0.79				-0.53			0.53		0.88	0.76	0.87	0.69	0.87									
Ni		0.68	0.85	0.87				-0.57			0.32		0.88	0.85	0.82	0.89	0.89	0.29	0.84							
PO_4 P				0.41					0.31				0.28	0.48		0.53	0.55									
NH_4 N																										
NO_3 N		-0.43	0.45	-0.42				0.45					-0.43	-0.38	-0.45	-038	-0.38		-0.37	-0.34						
SO_4 S		0.84	-0.74	0.57				-0.58	0.49	0.33	0.76	0.49	0.69	0.56	0.76	0.42	0.66		0.67	0.65			-0.39			
Cl		-0.44	0.62	-0.44				0.63			-0.27		-0.44	-0.39	-0.51	-0.35	-0.43		-0.43	-0.48			0.43	-0.57		
Si		0.69	-0.78	0.81				-0.53			0.30		0.84	0.76	0.80	0.75	0.81	0.42	0.78	0.86			-0.43	0.54	-0.46	

parameters which were found to be significantly correlated at and above the 95% confidence limit are given in Table 5.4.

As can be seen from the results, many ions were significantly correlated with each other above the 99% level. As found with the analysis of data from the two general surveys (see 4.7), many of the parameters varied inversely with pH and directly with acidity. There was also a much greater degree of correlation between pH and acidity ($r = -0.82$) than was found in the two general surveys; this was possibly due to the larger range of pH and acidity values examined in the Brandon Acid Stream system.

Conductivity was once again strongly correlated with those ions which were present in large concentrations and also those which tended to be the most variable, for example, pH ($r = -0.77$), acidity ($r = 0.66$), Mn ($r = 0.83$), Al ($r = 0.75$) and SO_4-S ($r = 0.84$).

In relation to the acidity values, it was thought that there was sufficient data to allow further statistical analysis to be carried out in an attempt to find which parameters were related in any way to the acidity of the waters. Therefore, a stepwise regression analysis was performed, with acidity as the dependent variable and all other parameters measured as the independent variables. The results of the analysis are given in Table 5.5 and show that Al, Fe and pH were highly correlated with acidity.

Table 5.5 Stepwise regression analysis of chemical data collected from Brandon Pithouse Acid Stream for the period of October 1972 to March 1975.

Dependent variable - acidity.

Variable	multiple r
aluminium	0.91532
iron	0.94422
pH	0.94773
temperature	0.95027
copper	0.95251
nickel	0.95310
magnesium	0.95358
sodium	0.95479
potassium	0.95539
phosphate phosphorous	0.95565
cobalt	0.95588
sulphate sulphur	0.95602
% oxygen	0.95616
nitrate nitrogen	0.95632
ammonium nitrogen	0.95644
chloride	0.95652
manganese	0.95657
lead	0.95663
calcium	0.95669
(constant)	

reach number	1	2	3	4	5	6	7	7b	8	9	10	11	12	13	14	15	16	17	18	19	[illegible]
number of samples	20	20	20	20	16	16	20	20	20	20	20	20	20	20	20	16	16	17	18	19	
species	I																				
Euglena mutabilis	20	20	19	19	15	14		16	10	17	13	11	6	1	1						2.6
Eunotia exigua	20	17	16	16	14	13	18	18	20	18	20	20	17	4	2	1	2				2.6
Nitzschia elliptica var. alexandrina		12	15	11	6	5			9	7	8	1									2.6
Pinnularia acoricola			2	3	1																2.6
Nitzschia subcapitellata	3	1		2			15	15	18	5	17	17	13	5	6						2.6
Chlamydomonas applanata var. acidophila	3		2	8	3	2	5	5	5	1	6	2	1	1							2.6
Gloeochrysis turfosa	10	2	5	9	9	4	6	5	8	4	10	15	4	4	3						2.6
Characium sp.	2			2	1	1	4	2	3	1	1	1	1	1							2.6
Stichococcus bacillaris	5			1		1								1							2.6
Hormidium rivulare	2	1	2	1	1	1	15	19	16	14	17	13	8	7	5						2.6
Drepanocladus fluitans (adult)	20		17	20	16	15	20	14	20	20	20	20									2.6
D.fluitans (protonema)	20	20			7	9			7		12										2.6
Dicranella palustris					2			20													2.6
Lepocinclis ovum									1		7	3	2	4	4						3.0
Nitzschia palea							2	2								1	8	1	3	3	2.9
Juncus effusus					16	16	20	17			20	20	20	20							2.6
							II														
Microthamnion strictissimum											1	1	1	2	1	1					3.1
Diatoma vulgare									3	2			2	1	8	12	2	1	10	11	5.2
Amphiprora sp.									2	1									1	2	5.6
Navicula gregaria									1	1			1	1	6	11	8	3	13	13	5.2
Navicula sp. (40x15μm)									2	2					7	6	2	2	13	12	5.5
Nitzschia sigmoidea													1	1		3	12		3	5	5.2
Surirella ovalis													1			4			1	3	4.8
Pinnularia guillarmodiae											1		1								4.8
Chlamydomonas sp.									1				1			4	1			2	4.2
Cryptomonas sp.											3	2	2	4	1		1	2			3.3
Oscillatoria pseudogeminata									2	1			1	1	2		5				5.8
Scapania undulata															20	12					4.7
Synedra ulna														2	8	12		3	4	6	5.8
Achnanthes minutissima															9	12	15	4	12	10	4.9
Achnanthes microcephala															2		11	2	12	10	5.6
Cocconeis placentula															1		4		10	11	6.2
Microspora lauterbornii															6	2	4				5.8
															III						
Navicula acicularis																	4	2	2	4	6.8
Navicula occulta																	2		1	1	7.5
Nitzschia acuta																	2				7.2
Cymbella ventricosa																	2	3	2	2	6.8
Pleurosigma sp.																	1	2			7.2
Gomphonema acuminatum																	2	1	2	3	6.8
Protococcus sp.																	5	2	2	3	6.8
Ankistrodesmus sp.																	1	1	2	1	7.2
Oscillatoria sp. (10μm)																	1		5	6	6.8
Lyngbya sp. (6μm)																	3		7	4	7.2
Chantransia																	8	1			6.8
Eurhynchium ripariodes																	12		14	12	6.8
																	IV				
Ulothrix moniliformis																		1			7.0
Cladophora glomerata																			12	12	7.0
Vaucheria sessilis																			8	10	7.0
Fontinalis antipyretica																			10	17	7.0
Mougeotia sp. (25μm)																		3			7.0
Sphaerocystis sp.																			3	2	7.3
Diatoma hiemale																			8	7	7.0
Rhoicosphenia curvata																			13	12	7.0
Nitzschia sp. (12μm)																		2			7.0
Pinnularia sp. (40μm)																		2		1	7.5
Melosira varians																			8	9	7.0
Gyrosigma sp.																			1	2	7.5
Navicula avenacea																			3	5	7.6
Caloneis sp.																			1	3	7.8

5.6 Distribution of photosynthetic organisms, for Brandon Pithouse Acid Stream, October 1972 - March 1975

5.61 Overall trends in distribution

The distribution of species down Brandon Acid Stream, from the source to the Redburn and from there to the confluence with the R. Deerness, are given in Table 5.6. The number of occasions the species were recorded at each reach and the number of times each reach was sampled for photosynthetic organisms, are also given in Table 5.6, together with the lowest pH value at which each species was recorded in the Brandon Pithouse Acid Stream system.

59 species were recorded over the period October 1972 to March 1975; of those, 53 were algae, 5 were bryophytes (*Drepanocladus fluitans* was recorded separately as adult and protonema), and one an angiosperm. The maximum, minimum and mean number of species present at each reach are shown in Fig. 5/25. This summary demonstrates the general increase in species number with an increase in pH and a decrease in concentration of the acid mine water. From reaches 1 to 15 the number of species present at a reach was relatively stable, with the largest variation occurring at reaches 10 and 11, where there was a range of 4 to 8 species over the period of study. The mean number of species increased from 5 to 15, between reach 15 in the acid stream and reach 16 in Redburn. The highest mean of 19 species occurred at reaches 18 and 19 in the R. Deerness. The lowest number of species were recorded at reach 13, where on several occasions only 2 species were found.

5.62 Stream A, reaches 1-6 from October 1972 - March 1975

The number of species remained reasonably constant and low over reaches 1-6 for the period of study. Reach 2 had consistently the smallest number of species, whilst the greatest number was recorded at reach 1. The flora of reaches, 1, 4 and 5 were dominated by the adult moss Drepanocladus fluitans, whilst the alga Euglena mutabilis was present at all the reaches and was invariably the dominant alga present. It was particularly abundant at reach 1 often covering over 60% of the substratum.

The protonema of the moss Drepanocladus fluitans dominated reach 2, probably because it was capable of withstanding the relatively fast current speeds which were characteristic of this reach. The stable substratum and the protection afforded against the fast current speeds by the filaments of protonema, also encouraged the growth of Euglena mutabilis and to a lesser extent Eunotia exigua. At reach 3, the algal flora ceased to be dominated by Euglena mutabilis and although the species remained abundant, the diatoms Nitzschia elliptica var. alexandrina and Eunotia exigua tended to dominate the population. The substratum which consisted mainly of small pebbles appeared to be unsuitable for the colonization of the moss protonema. However, small growths of the adult moss were present in the slower currents to the side of the stream.

Although the bed of the holding reservoir was covered with adult Drepanocladus fluitans, throughout the study, the protonema was never recorded in the reservoir itself. The population was similar to other reaches and extensive growth of Euglena mutabilis and diatoms occurred at the edge of the reservoir. This was the only reach where Pinnularia acoricola and Chlamydomonas applanata var. acidophila were present in large numbers and on occasions during the summer, the chlamydomonad was found to form large blooms.

5.63 Stream B, reaches 7 and 7b, October 1972 - March 1975

Although the flora of stream B was similar to stream A (reaches 1 - 6) as regards composition of species, there were differences in the frequency of occurrence and the abundance of the species. The only species that was present in stream B, but not A, was Nitzschia palea, and this was only found on two occasions. Stichococcous bacillaris and Nitzschia elliptica var. alexandrina were recorded in stream A , but were absent from stream B. Like several reaches of stream A, the reaches 7 and 7b were dominated by the adult form of Drepanocladus fluitans. The other moss recorded in the acid streams was Dicronella palustris and although it was not as prolific as Drepanocladus fluitans, it tended to dominate the area of acid seepage at the source of stream B (reach 7b). The algal flora at reach 7b was dominated by Hormidium rivulare and the diatoms Nitzschia subcapitellata and Eunotia exigua. The first two species listed above were also present in stream A but were never as abundant as they occurred at reach 7b.

In contrast, Euglena mutabilis was more abundant in stream A than stream B.

Although reach 7 was at a higher pH than reach 7b (Fig. 5.3) and also had a potential source of different species from the non-acid tributary which entered the stream below 7b, the flora was very similar to that of the more acid reaches. The main difference between the two was that Euglena mutabilis and Dicranella palustris were absent from reach 7.

5.64 Downstream of the confluence, reaches 8 - 16, October 1972 - July 1974

At reach 8 and to a lesser extent reach 9, the number and abundance of species was reduced compared with reaches above and below the confluence. This reduction was thought to be due to the presence of the iron oxide precipitate previously mentioned. Further reference to the effects of the precipitate are given in 8.71. Besides the adult moss, Drepanocladus fluitans, which dominated reach 8 and 9, the diatoms Eunotia exigua and Nitzschia subcapitellata, and Hormidium rivulare were the most commonly occurring and abundant species. However, their growth at these reaches was always reduced compared with other reaches which were lacking the precipitate.

The mean number of species at reaches 10 and 11 increased from 4 to 6 and again the Drepanocladus fluitans was the most abundant, submerged photosynthetic organism. The algal flora was again dominated by the same species as found at reach 8, although their abundance was greater. Gloeochrysis turfosa was also common at these reaches, often forming a thin film of cells over the surface of the water away from the faster currents. The angiosperm, Juncus effusus was prolific at the edge of the water for this section of the stream and in many places was actually growing in the water, as it did in reach 7b. From reach 8 downwards, the occurrence of Euglena mutabilis was less frequent.

As already mentioned in 5.61, the least number of species occurred at reach 13, where iron oxide precipitate was especially heavy. Of these, Eunotia exigua, Hormidium rivulare and Gloeochrysis turfosa were the most commonly occurring species, although they were rarely macroscopically obvious. At reaches 13, 14 and 15, several species occurred which were not recorded at lower pH values further up the stream. Diatoma vulgare, Navicula gregaria, Synedra ulna and Achnanthes minutissima were particularly common, especially at reach 14. At this reach Scapania undulata was present for the total period of the study and also became established at reach 15 during the same period.

As can be seen from Fig. 5/25 the mean number of species occurring in Redburn below the confluence with the acid stream was greater than at any reach in the acid stream. Several species that were recorded in the acid stream were also found in Redburn and the R. Deerness; these included, Diatoma vulgare, Navicula spp. and Achnanthes spp.. Eunotia *exigua* was the only species recorded both in the lowest pH values of the acid stream and in the Redburn.

5.65 Reaches 8 - 16, July - November 1974

During the period of high pH values (see 5.42), the composition of the algal flora changed considerably at several reaches. The most noticeable of these were at reach 8, where the population changed from species capable of growth below pH 3.0, to species commonly found in less acid waters. The first species to occur and grow prolifically at reach 8, which were not present before the increase in pH were *Diatoma vulgare*, *Amphiprora* sp. and *Oscillatoria pseudogeminata*. Although these species dominated the algal population during this period, several of the previous inhabitants of the reach remained present, but in smaller numbers (see Table 5.7). Of these, *Eunotia exigua* and *Nitzschia subcapitellata* were the most common.

At reaches 10 and 11 where the pH change was buffered by the acid seepage (5.36), the composition of the algal population remained fairly constant, although *Microthamnion strictissimum* colonised an area where previously it had been absent. Those species which dominated reach 8, during this period also increased in abundance at lower reaches, in particular, reaches 13 and 14. Again, *Diatoma vulgare* dominated the population and at reach 14 often formed macroscopically obvious growths.

5.66 Reaches 8 - 16, November 1974 - March 1975

Following the return to low pH conditions after

Table 5.7

Comparison of algal floras at reach 8, for the different pH regimes from October 1972 to March 1975

	October 1972 to July 1974	July to November 1974	November 1974 to March 1975
number samples	10	5	5
species			
Euglena mutabilis	5		5
Eunotia exigua	10	5	5
Nitzschia elliptica var. *alexandrina*	4		5
Chlamydomonas applanata var. *acidophila*	4		1
Gloeochrysis turfosa	5	1	2
Characium sp.	3		
Hormidium rivulare	9	3	4
Drepanocladus fluitans adult	10	5	5
D. fluitans protonema	2		5
Lepocinclis ovum		1	
Diatoma vulgare		5	
Amphiprora sp.		4	
Navicula gregaria		5	
Chlamydomonas sp.		2	
Oscillatoria pseudogeminata		4	

November 1974, the algal community at reach 8, rapidly returned to it's previous composition (see Table 5.7). The species which had dominated the population at the higher pH, were found to die within a few days of the decrease in pH to 3.0 and within three weeks there was an obvious increase in numbers of Eunotia exigua, Nitzschia subcapitellata, Euglena mutabilis and moss protonema. The latter two species were particularly abundant, especially the protonema whose occurrence at this reach had been previously rare.

At reaches 10 and 11, as with the chemical parameters, there was little change in the species composition, although both the Hormidium rivulare and moss protonema increased in abundance.

At reaches 13 to 16, the number of species decreased markedly because of the decrease in pH, from a mean value of pH 6.0 to below pH 5.0 and also because of the large increase in iron oxide precipitate which appeared over this section of the stream (see 5.53). At reach 14, Diatoma vulgare, Navicula gregaria and Scapania undulata survived the change in conditions, but the growth of all three species was reduced. There was also an increase in the occurrence of the more acid tolerant species such as Eunotia exigua and Gloeochrysis turfosa.

5.7 Ordination of species according to tolerance to acid mine water

It was possible to ordinate the species found in the

Brandon Acid Stream system into four groups on the basis of their tolerance to acid mine water. As previously mentioned and demonstrated in Table 4.1, the species growing in the acid waters are subjected to quite large concentrations of heavy metals as well as low pH and high acidity. The species given in Table 5.6 are presented in groups I to IV, in order of their tolerance to Brandon Pithouse Acid Stream water.

5.71 Group I

The species presented in Group I were those organisms which were capable of growing over the pH range 2.6 to 3.0 and also in the greatest concentration of heavy metals present in the acid stream. All these species, with the exception of Lepocinclis ovum and Nitzschia palea, were recorded at pH 2.6. However, with the exception of Pinnularia acoricola, all the species in this group were not restricted to growth in the pH 2.6 to 3.0 range. The species which were more common in the more acidic reaches 1 to 6, included Euglena mutabilis, Nitzschia elliptica, Stichococcus bacillaris, Drepanocladus fluitans protonema; in contrast Hormidium rivulare, Nitzschia subcapitellata, N. palea, and Lepocinclis ovum were recorded mainly in the less acidic reaches, for example 7, 7b, 8 to 11. Eunotia exigua was the only species which was recorded present on at least one occasion in every reach of the acid stream (reaches 1 to 15); it was also the only organism in Group I, except Nitzschia palea, which was found in Redburn. The flagellated species Chlamydomonas *applanata* and *Gloeochrysis turfosa* were also distributed over a wide pH, acidity and heavy metal range.

5.72 Group II

The species included in this group were present in the less acid reaches of the stream (reaches 12 to 15) between pH 3.0 and 6.0. Those species which colonized reaches 8 - 15, when the pH increased during July to November 1974, are also presented in Group II. Many of these species were recorded between pH 4.5 to 5.5 but did not survive at pH values of less than pH 4.0 during the November 1974 to March 1975 period. *Microthemnion strictissimum* and the cryptomonad species were the only species to grow below pH 4.0. With the exception of *M. strictissimum* and *Scapania undulata*, all other species were also recorded in Redburn and the R. Deerness. Although the pH value increased and the acidity decreased in the lower reaches of the stream the species were still subjected to relatively large concentrations of heavy metals and large amounts of iron oxide precipitate. *Oscillatoria pseudogeminata* was the only blue-green alga recorded which grew in abundance below pH 6.0.

5.73 Group III

These species were recorded above pH 6.0 and only in the Redburn or the R. Deerness. They were capable of tolerating some deposition of iron hydroxide and quite high levels of heavy metals. The chantransia - of *Lemanea* sp. was the only organism which was present in abundance in Redburn at reach 16 and which was not found in the R. Deerness.

5.74 Group IV

The species in Group IV were those which were only recorded at reach 17, lower down the Redburn, and above and below the confluence in the R. Deerness. No species were present in the upstream reach (18) of the R. Deerness that were not found downstream of the confluence. However, several species were more commonly found in the downstream reach.

5.75 Statistical analysis for data concerning number of species

Pearsons correlation coefficients were computed for all parameters measured against the number of species (Table 5.4). Similar analysis has, as yet, not been completed for individual species as more data are needed to gain meaningful results.

As can be seen from Table 5.4, the number of species at a reach was correlated significantly with several parameters. The highest correlation was between pH and the number of species ($r = 0.71$, $p \simeq 0.001$) whilst other parameters that were significant above the 99% limit included conductivity, acidity, temperature, Mg, Zn, Cu, Mn, Fe, Al, Co and Ni. There was also a correlation between some of the anions (not PO_4-P and NH_4-N) and the number of species.

As so many parameters were significantly correlated with number of species, stepwise regression analysis was performed on the data in order to try and ascertain which parameters had the greatest interaction with the number of

Table 5.8 Stepwise regression analysis of data collected from Brandon Pithouse Acid Stream.

Dependent variable - number of species.

Variable	multiple r	r squared	rsq change	simple r	B	beta
pH	0.70415	0.49583	0.49583	0.70415	1.40791	0.50276
temperature	0.74786	0.55930	0.06347	0.28210	0.64377	0.33628
chloride	0.79053	0.062493	0.06563	0.62033	0.04806	0.13428
nitrate	0.80430	0.64691	0.02197	0.46134	1.10570	0.14272
phosphate phosphorous	0.81580	0.66553	0.01862	0.10353	2.98852	0.07595
nickle	0.81933	0.67130	0.00577	-0.56000	11.02449	0.39831
% oxygen	0.82176	0.67529	0.00399	0.15331	0.03522	0.05133
sulphate sulphur	0.82410	0.67914	0.00385	-0.58243	-0.01153	-0.45969
magnesium	0.83494	0.69713	0.01799	-0.27590	0.04106	0.23681
manganese	0.83888	0.70372	0.00659	-0.61659	-0.83208	-0.40652
aluminium	0.84173	0.70851	0.00479	-0.53479	-0.11366	0.26311
silicate	0.84392	0.71221	0.00370	-0.52444	-0.06312	-0.19667
conductivity	0.84658	0.71670	0.00450	-0.46761	0.00158	0.16195
ammonium nitrogen	0.84744	0.71815	0.00144	0.10776	0.60432	0.04495
copper	0.84774	0.71866	0.00025	-0.48766	-2.52246	-0.13095
potassium	0.84799	0.71909	0.00043	0.08999	0.00731	0.05156
zinc	0.84829	0.71960	0.00051	-0.55144	1.74275	0.14754
iron	0.84855	0.72004	0.00004	-0.43079	0.00503	0.04158
acidity	0.84858	0.72010	0.00005	-0.50631	-0.00034	-0.02415
(constant)						

species. The results in Table 5.8 show that of the 25 independent variables tested, the parameters which had the greatest influence on the number of species at a reach, were pH and water temperature.

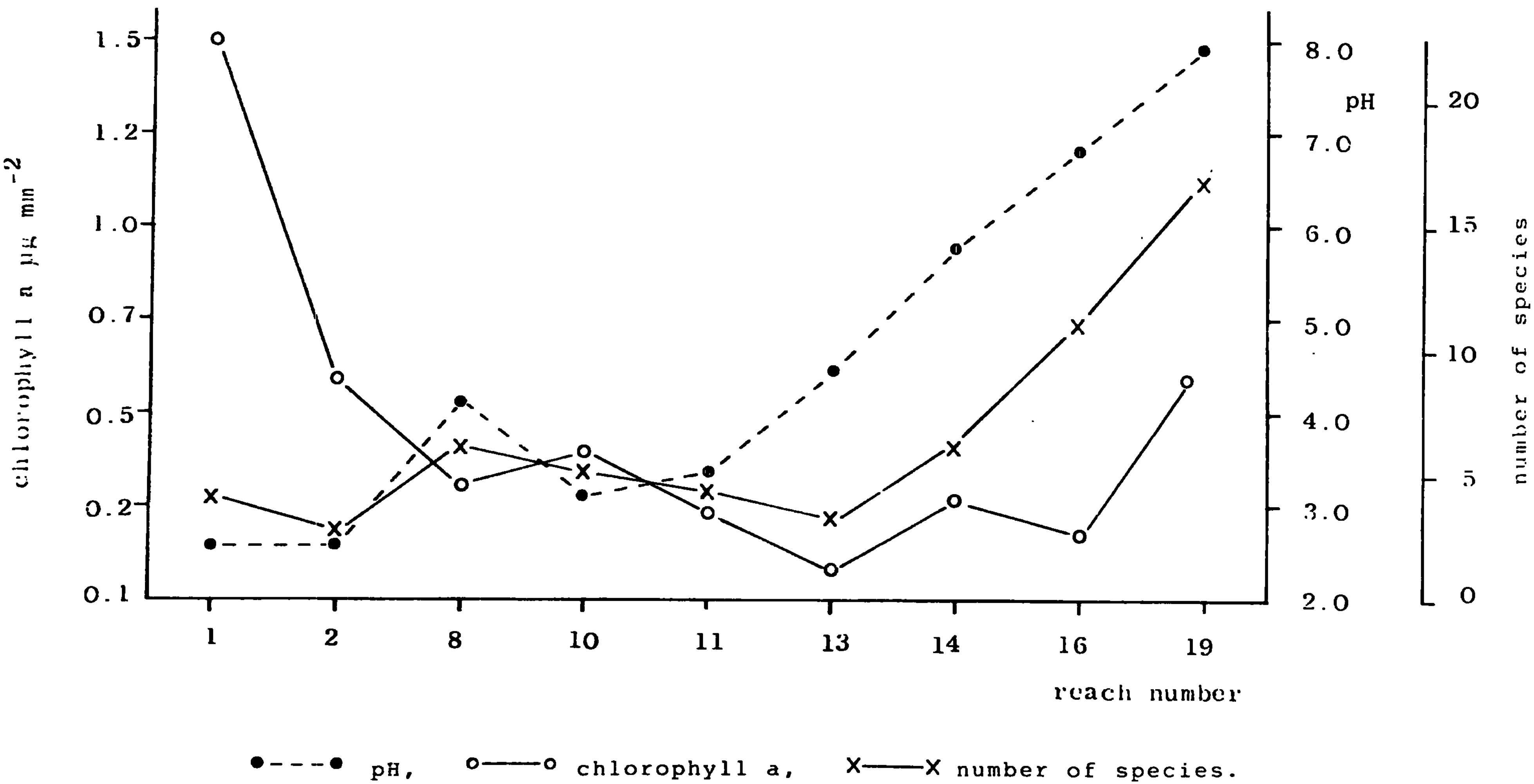
5.8 Maximum standing crop of algae at selected reaches down the Brandon Pithouse Acid Stream

The maximum standing crop (M.S.C.) of algae, expressed as chlorophyll a $\mu g\ mm^{-2}$, was determined at monthly intervals for 9 reaches down the stream (Table 2.2). The pH and number of species present in each reach were also determined and are given in Fig. 5/28 and Fig. 5/29. These values have also been incorporated in results for Fig. 5, 5.3 and 5/25.

5.81 Mean values for all reaches over a 12 month period

Fig. 5/28a gives the mean values of the maximum standing crop, the mean pH and the mean number of species, for the 12 samples taken during the period July 1974 to June 1975. The mean values demonstrate the variation in the standing crop down the pH gradient, as well as indicating an apparant relationship between the three parameters measured. The highest mean M.S.C. value of 1.5 $\mu g\ mm^{2}$ occurred at reach 1 and the lowest value of 0.5 $\mu g\ mm^{2}$ at reach 13. From reaches 1 to 15 there was a gradual decrease in the mean M.S.C. value as the pH of the water increased and it was only at reach 19 in the R. Deerness that the mean value increased significantly, to approach the values obtained in reach 1.

Fig. 5/28 The mean values of 12 monthly samples for maximum standing crop, pH and number of species, for 9 10 m reaches of Brandon Pithouse Acid Stream.



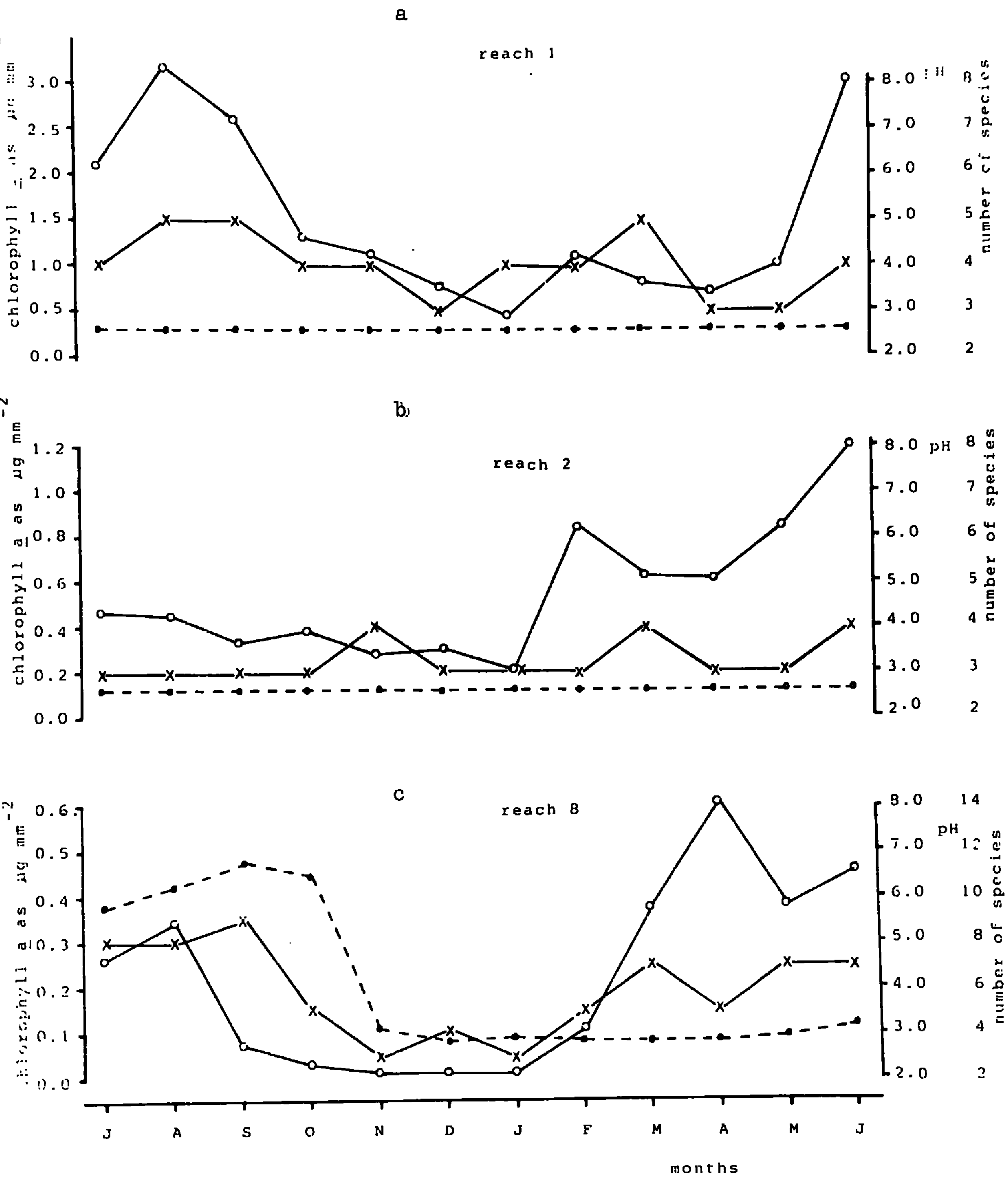
The relationship between the M.S.C. and number of species, indicated that the standing crop values fluctuated with changes in species number. At reach 13 where, as previously mentioned, the iron hydroxide precipitate was particularly abundant, the M.S.C. was considerably reduced..

5.82 Maximum standing crops for reach 1

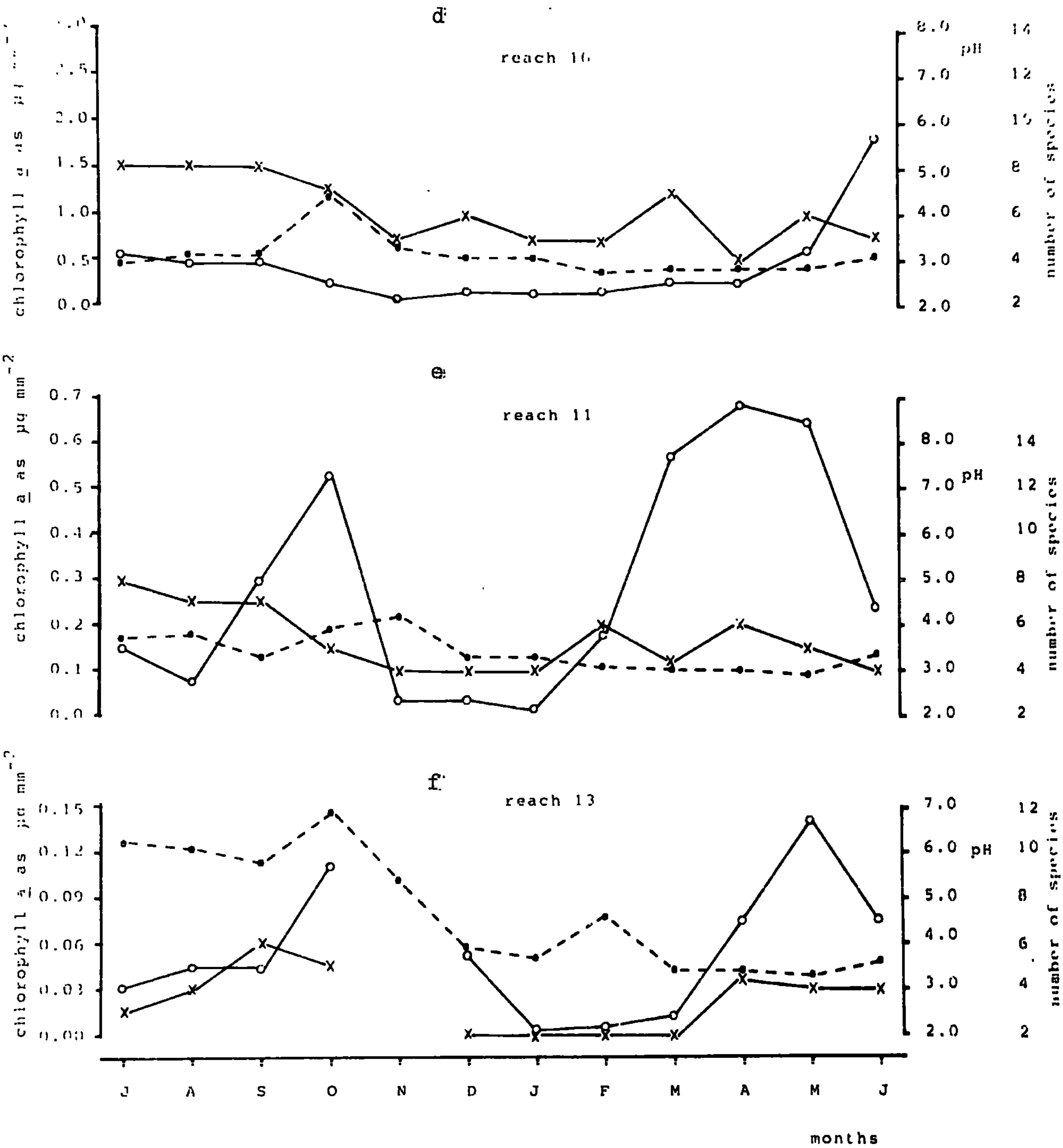
The individual monthly results for maximum standing crop, pH and number of species, are given in Fig. 5/29a. The results show that this reach had the highest mean M.S.C. value and also the highest individual monthly value of 3.2 $\mu g\ mm^{-2}$ chlorophyll a. The general pattern shown at reach 1 over the 12 months, was typical of other reaches, with the maximum values occurring from June to late September, followed by a gradual decrease to a minimum value in January. The increase which was recorded in the late winter, was due mainly to a bloom of *Eunotia exigua*, whilst the larger summer values were as a result of extensive growth of *Euglena mutabilis*. Other species which contributed towards the large M.S.C. values were *Gloeochrysis turfosa* and the moss protonema.

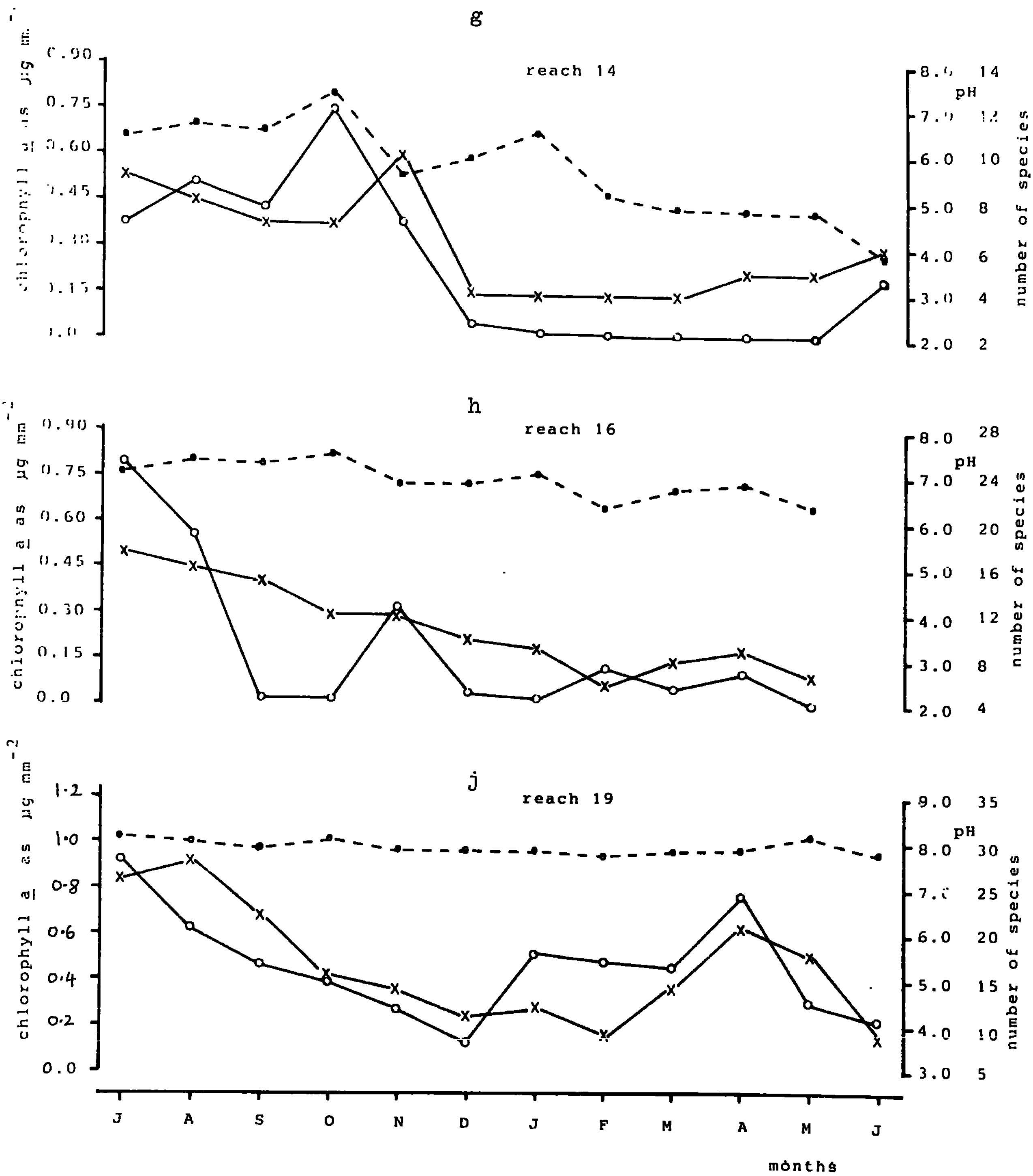
5.83 Reach 2

Although the pH, water chemistry (Fig. 5.1 - 5/27) and number of species present at this reach were similar to reach 1, the M.S.C. only attained a maximum value of 1.2 $\mu g\ mm^{-2}$ chlorophyll a (Fig. 5/29). As mentioned in 5.62, this reach was covered by moss protonema throughout the year and therefore each sample taken contained a high proportion of the protonemal filaments. From February



●- - -● pH, o——o chlorophyll $\underline{a}$ ug mm^{-2}, X——X number of species


d
reach 16
chlorophyll a as µg mm⁻²
pH
number of species
e
reach 11
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
14
12
10
8
6
4
2
f
reach 13
0.15
0.12
0.09
0.06
0.03
0.00
J A S O N D J F M A M J
months


g
reach 14
chlorophyll a as µg mm⁻²
0.90
0.75
0.60
0.45
0.30
0.15
0.0
8.0 14
pH
7.0 12
6.0 10
5.0 8
4.0 6
3.0 4
2.0 2
number of species
h
reach 16
chlorophyll a as µg mm⁻²
0.90
0.75
0.60
0.45
0.30
0.15
0.0
8.0 28
pH
7.0 24
6.0 20
5.0 16
4.0 12
3.0 8
2.0 4
number of species
j
reach 19
chlorophyll a as µg mm⁻²
1·2
1·0
0·8
0·6
0·4
0·2
0.0
9.0 35
pH
8.0 30
7.0 25
6.0 20
5.0 15
4.0 10
3.0 5
number of species
J
A
S
O
N
D
J
F
M
A
M
J
months

to June the M.S.C. increased due to greater growth of the protonema and a higher density of Euglena mutabilis and diatoms in association with the moss. The current speed (Fig. 5/26) and the amount of scouring were high at reach 2 and therefore, the increased biomass of protonema offered greater protection to the algae against the faster currents.

5.84 Reach 8

The M.S.C. values from July to October (Fig. 5/29c) were taken when the pH at reach 8 had increased (see 5.42). During this period, the number of species present were not only different to the pre-July period (see 5.64), but were also more numerous, increasing from a mean of 4 to that of 8 algal species. The increase in number of non acidic species coincided with an increase in the standing crop from 0.26 $\mu g\ mm^{2}$ to 0.35 $\mu g\ mm^{-2}$ chlorophyll a. This increase was attributed to Diatoma vulgare and Oscillatoria pseudogeminata. The increased standing crop during this period was partly attributed to a decrease in the amount of iron oxide precipitate (see 5.42).

In November, the M.S.C. as well as the pH, and number of species, decreased. The low M.S.C. values (0.02 $\mu g\ mm^{2}$) from November to January were due to some extent to seasonal variation, but also to changes in the flora. The increases which followed in February and March, were due mainly to the establishment of moss protonema and Euglena mutabilis and also to the reduction in iron oxide precipitate, mentioned in 5.51.

The values recorded in May and June were almost entirely due to an increase in growth of Hormidium rivulare.

5.84 Reach 10

The standing crop in reach 10 was consistent and comparatively high, compared with other higher pH reaches. These results (Fig. 5.29/d) characterize the seasonal variation in M.S.C. at a reach where physical and chemical conditions were relatively stable. The maximum value of 1.8 μg mm^2 chlorophyll a was obtained during June for a population of Hormidium rivulare. The results obtained in the spring were representative of a crop of Eunotia exigua and Nitzschia subcapitellata, whilst during the late winter months when the pH dropped below pH 3.0, the amount of protonema increased.

As with reach 1, the amount of scouring and the variation in water chemistry was less for this reach than others in this section of the stream. Nevertheless, the M.S.C. values were consistently lower than those of reach 1, indicating the effects of increased competition amongst the algae and a possible increase in predation in the less acid conditions.

5.85 Reach 11

Although the same species were present at reaches 10 and 11 the standing crop was generally lower at reach 11 (Fig.5/28 and Fig. 5/29e). The highest values recorded in June were due to Hormidium rivulare and Eunotia exigua, whilst the peak in September was mainly due to Nitzschia *subcapitellata*. A similar seasonal pattern was recorded at this reach as was found for the other reaches.

The lower values obtained at this reach could not be explained, but it was possible that some nutrient which had not been detected was being removed by the bed of moss upstream of this reach. Increased predation may also have contributed to the lower values.

5.86 Reach 13

As mentioned in 5.81, the results recorded at reach 13 (Fig. 5/29f) demonstrate the effect of large quantities of flocculent iron oxide precipitate. The values obtained were generally lower than at any other reach, with a maximum value of 0.15 µg mm^{-2} and a minimum of only 0.002 µg mm^{-2} chlorophyll *a*. Even though growth at this reach was very restricted, the same seasonal cycle was observed. The increased values from April to June were due to *Eunotia exigua* and *N. subcapitellata*, whilst the crop from July to November were mainly due to *Microthamnion strictissimum* and *Diatoma vulgare*.

5.87 Reach 14

From July to November, when the pH of the stream was at its highest, the M.S.C. value at this reach was greater than at any other reach on the lower half of the stream (12-16). These values (Fig. 5/29g) were obtained mainly as a result of the growth of *Diatoma vulgare* and *Microspora lauterbaunii* (see Table 5.6).

The sudden decrease in December was due, in part, to seasonal variation and also to large amounts of iron oxide deposition. This situation persisted for the rest of the study period. The only significant increase in M.S.C. was attributed to growth of Hormidium rivulare, which seemed capable of restricted growth under these conditions.

5.88 Reach 16

Below the confluence of the acid stream in Redburn, the standing crop of algae was reduced. The largest M.S.C. of 0.75 $\mu g\ mm^{-2}$ occurred in July and consisted mainly of blue-green algae and Lemanea sp.

The increase in the iron oxide deposit in December through to May, caused a decrease in the M.S.C. value. A comparison of the growth of algae in this reach with reach 19, demonstrates the effect near-neutral mine water has on the standing crop of algae. At reach 19 the M.S.C. only fell below 0.5 $\mu g\ mm^{-2}$ chlorophyll a on one occasion, whereas at reach 16, the same value was only attained on two occasions and these were when the iron deposit was at a minimum.

5.89 Reach 19

At reach 19 (Fig. 5/29j) where the acid stream water was considered to have no measurable effect on the species, the standing crop was generally higher than any other reach, except reach 1. The seasonal pattern shown elsewhere was similar at this reach, with the maximum values being obtained in the summer when growth of Cladophora glomerata was at its greatest. The decrease in M.S.C. and species number in May and June was attributed to scouring by summer spates.

5.9 Statistical analysis

Pearson's correlation coefficients were determined for maximum standing crop, pH and number of species. The results of these analyses are given in Table 5.9. It can be seen that for the stream as a whole, there was significant, negative correlation, at the 99.9% level, between maximum standing crop and pH. Although the number of species and pH were similarly correlated (r = 0.752), there was no significant correlation between the M.S.C. and the number of species, when the whole stream was considered.

In order to determine which parameter was most likely to influence the M.S.C. , partial correlation coefficient analysis was carried out on the data. The results indicated that the relationship between pH and the M.S.C. was stronger than the relationship between number of species and M.S.C. The analysis also demonstrated that if the influence of pH is not considered in the relationship between M.S.C. and number of species, then the latter two parameters are more strongly correlated (partial correlation p =0.001 ordinary correlation p = 0.1).

This suggestion was supported by the Spearman's rank correlation analysis which was carried out on the data for the individual reaches (see Table 5.9). These results showed that for individual reaches where the pH was relatively constant throughout the 12 month collection period, a better correlation was observed between M.S.C. and number of species, than between M.S.C. and pH. Spearman's rank

	maximum standing crop v. pH	maximum standing crop v. number of species	number of species v. pH
Pearsons correlation coefficient(r)	-0.362	0.153	0.752
p =	0.001	0.1	0.001
Partial correlation coefficient	-0.669	0.623	0.831
p =	0.001	0.001	0.001

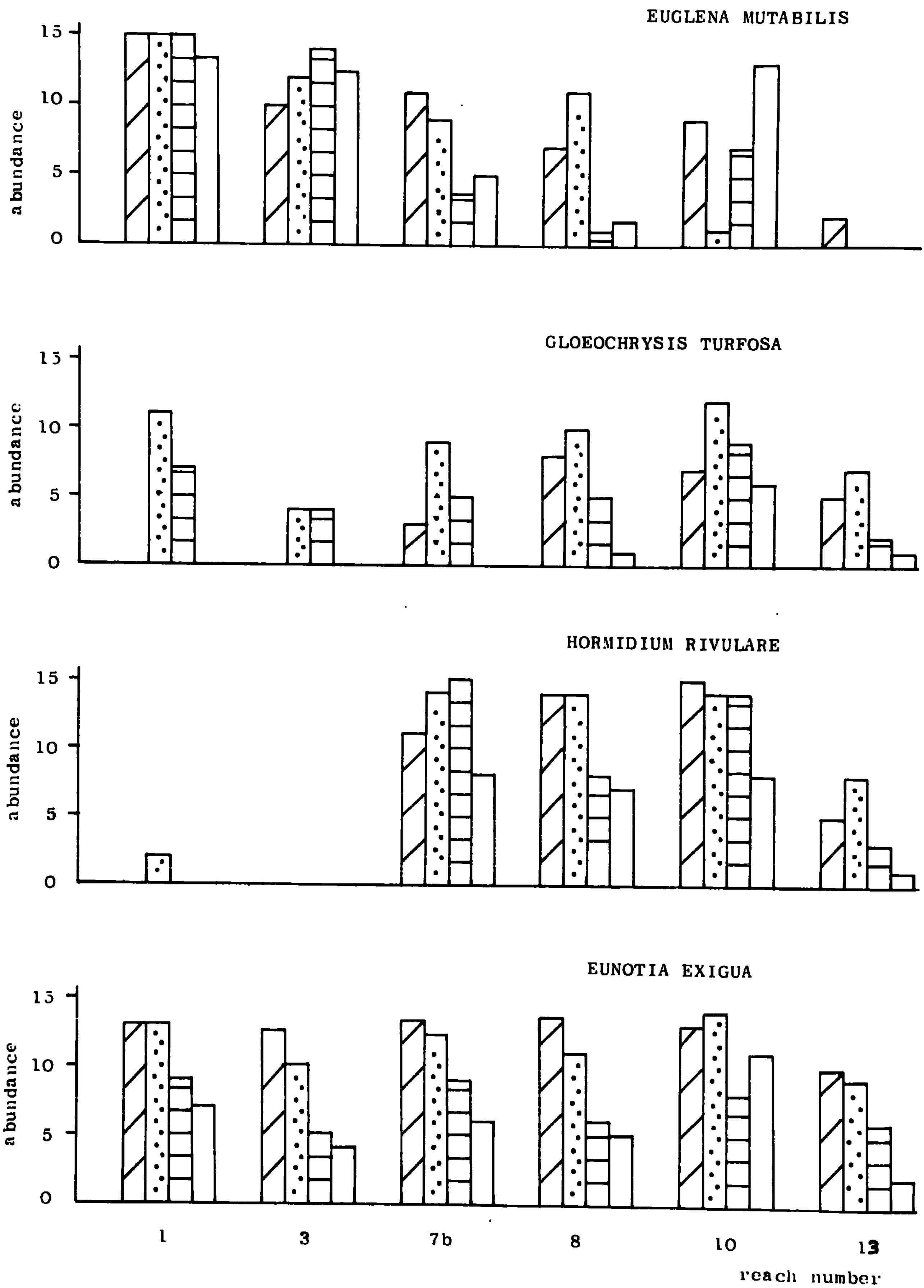
	max. standing crop v. pH		max. standing crop v. no. species	
reach number	Spearmans rank correlation (rs)	p =	Spearmans rank correlation (rs)	p =
1	*		0.572	0.05
2	*		0.332	< 0.1
8	-0.09	< 0.1	0.521	0.1
10	-0.603	0.02	0.676	0.01
11	-0.453	< 0.1	0.325	< 0.1
13	-0.19	< 0.1	0.631	0.02
14	0.724	0.001	0.704	0.01
16	0.184	< 0.1	0.334	< 0.1
19	0.162	< 0.1	0.503	0.1

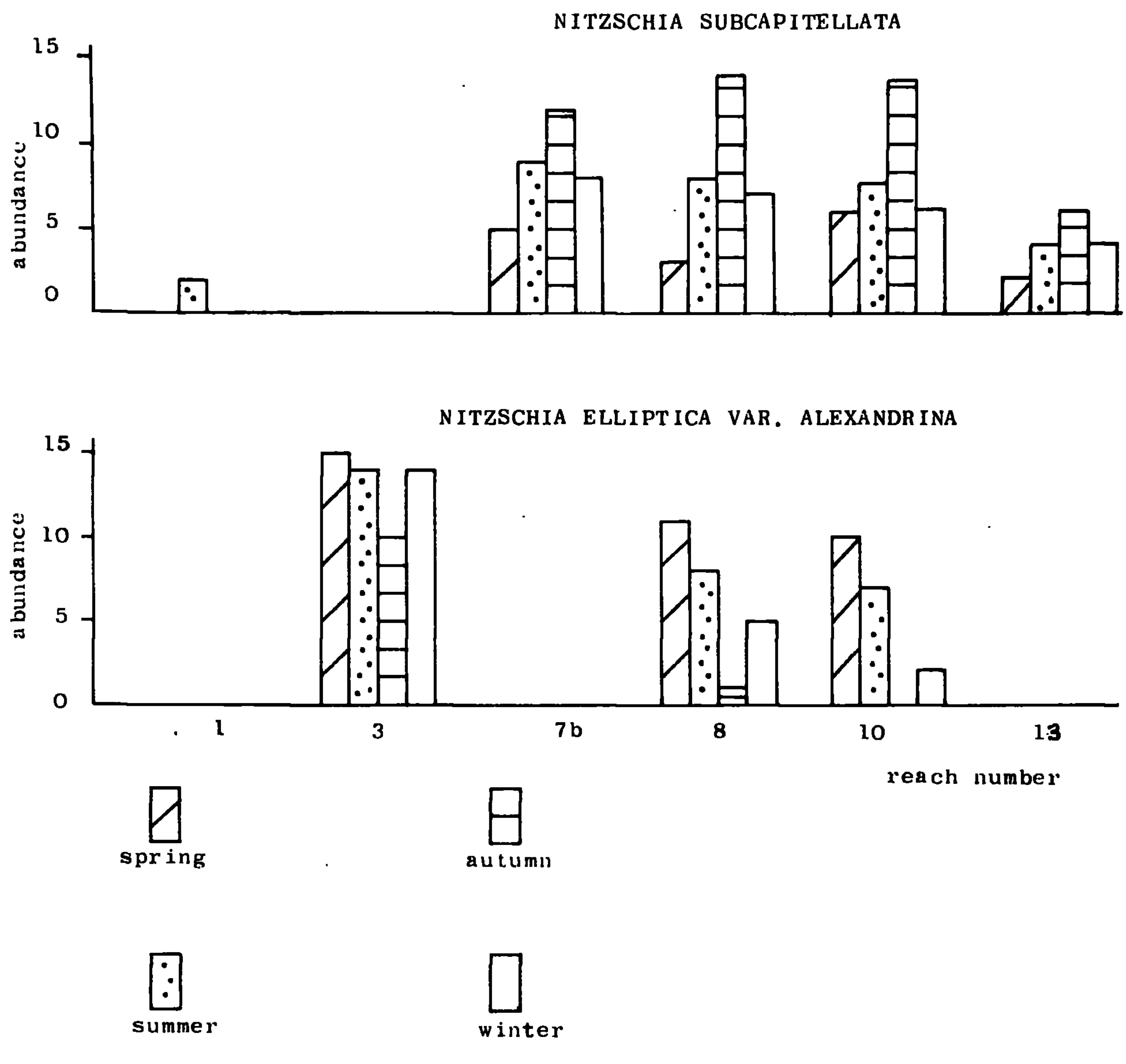
(* = no ranking possible because of too many tied pairs) correlation was used for the analysis of the data relating to the individual reaches because insufficient values were available in order to perform parametric analysis using Pearson's correlation.

5/10 Seasonal variation and succession of algal species

It was possible to show the succession and seasonal variation of several frequently occurring algal species down Brandon Pithouse Acid Stream, by comparing the relative abundance of the different species over the year. The amalgamated relative abundance values for six species, at six representative reaches over the four seasons, are given in Fig. 5/30. The abundance values given for each season comprize an amalgamation of three separate abundance scores, with each score being estimated on the basis of a 1-5 scale of relative abundance. Thus, if a species was the most abundant or equally abundant species in a reach for the three monthly samples, then the maximum score of 15 would be recorded for that season. Further discussion of the use of this technique is given in 2.53 and 8.33. Where the adult and protonemal stages of the moss were present, they were not considered in the estimations of abundance for the purpose of demonstrating seasonal variation, because both populations were reasonably stable throughout the year once they were established in a reach.

The seasonal variation of the individual species demonstrated in Fig. 5/30, showed that with the exception of Euglena mutabilis, the same general seasonal pattern was


EUGLENA MUTABILIS
GLOEOCHRYSIS TURFOSA
HORMIDIUM RIVULARE
EUNOTIA EXIGUA
abundance
0
5
10
15
1
3
7b
8
10
13
reach number


NITZSCHIA SUBCAPITELLATA
NITZSCHIA ELLIPTICA VAR. ALEXANDRINA
abundance
0
5
10
15
1
3
7b
8
10
13
reach number
spring
autumn
summer
winter

observed for the same species at different reaches. Euglena mutabilis showed a slightly different seasonal pattern at all six reaches and seemed, at least in the higher pH reaches (eg. 10), to be abundant mainly when other species were not at their maximum growth. At the lower pH values (reaches 1 and 3) however, the organism was usually the most abundant throughout the year.

Although the majority of the species tended to be present in varying abundance at a reach throughout the year, there was some succession of species demonstrated. Eunotia exigua and Nitzschia subcapitellata were generally found to be abundant at different seasons. Likewise, N. elliptica tended to be more abundant when the growth of N. subcapitellata was reduced. Eunotia exigua and Nitzschia elliptica were most abundant in spring and summer, whilst the N. subcapitellata was found to be more abundant in the autumn.

Hormidium rivulare demonstrated it's ability to grow successfully at all seasons except winter. Even at reaches 7b and 10 where it was usually most prolific, it was not abundant during the winter. Although Gloeochrysis turfosa rarely totally dominated the flora of a reach, it was most abundant at all reaches in the summer months and was rarely found in the winter. During the summer months it tended to compete with Euglena mutabilis, particularly at the higher pH value of reach 10 where it was in its greatest abundance. The seasonal variation of those and other species based on the data collected from Brandon Pithouse Acid Stream and the general surveys, are also dealt with in Chapter 7.

6. EXPERIMENTAL STUDIES

6.1 Introduction

This chapter reports a series of laboratory experiments carried out in an attempt to examine the influence of pH on the growth and morphology of five species of algae isolated from Brandon Pithouse Acid Stream. These include Chlamydomonas applanata var. acidophila, Euglena mutabilis, Gloeochrysis turfosa, Hormidium rivulare, and Stichococcus bacillaris. Further tests were also carried out on the growth of Euglena mutabilis and Hormidium rivulare to determine the effects of Zn and Cu (H. rivulare only) over a range of pH values. The adaptation of the species H. rivulare to pH was also briefly examined, as was the influence of various elements known to antagonize the toxicity of Zn to this species at higher pH values (Say & Whitton, in press).

The effect of temperature on the growth of four of the populations, at their optimum pH, was also examined. The term population, as used above, refers to the material isolated from one population in the field.

6.2 Preliminary investigations

Several preliminary investigations were made in order to ensure that the methods were standardized as much as possible.

6.21 Improvement in growth rate of Euglena mutabilis

An effort was made to improve the growth of some populations, in particular E. mutabilis which seemed to have a slower growth rate in the culture medium than the other species. A variety of natural and artificial substrata were introduced into the flasks containing E. mutabilis. These included stream mud, clay, soil, sand, gravel, glass beads, polystyrene beads and etched glass slides. The stream mud and sand were the only substrata which visually improved the growth of the species and therefore bi-phasic stock cultures were set up. However, these substrates could not be included in the experimental flasks as they would lead to changes in the chemistry of the media which would be difficult to standardize.

Field observations indicated that E. mutabilis was sensitive to strong light, and therefore rudimentary tests were carried out on light intensity and day length. The results showed that altering the day length did not significantly improve growth, but that a reduction in light intensity from 2000 lx to 1000 lx did bring about a significant improvement. As a result, all experiments on E. mutabilis were carried out at 1000 lx, whereas all the other populations were illuminated at 2000 lx.

6.22 Effect of varying sources of acid and alkali added to the media

An experiment was carried out to test the effect of changing the sources of acid and alkali used for pH adjustments to the media. As reported in 2.72, the pH of the culture medium was corrected by the addition of 1 N and 0.1 N H_2SO_4 and NaOH. In order to determine whether the type of acid or alkali providing the H^+ and OH^- ions to the medium had any effect on growth, HCl and KOH were substituted for the H_2SO_4

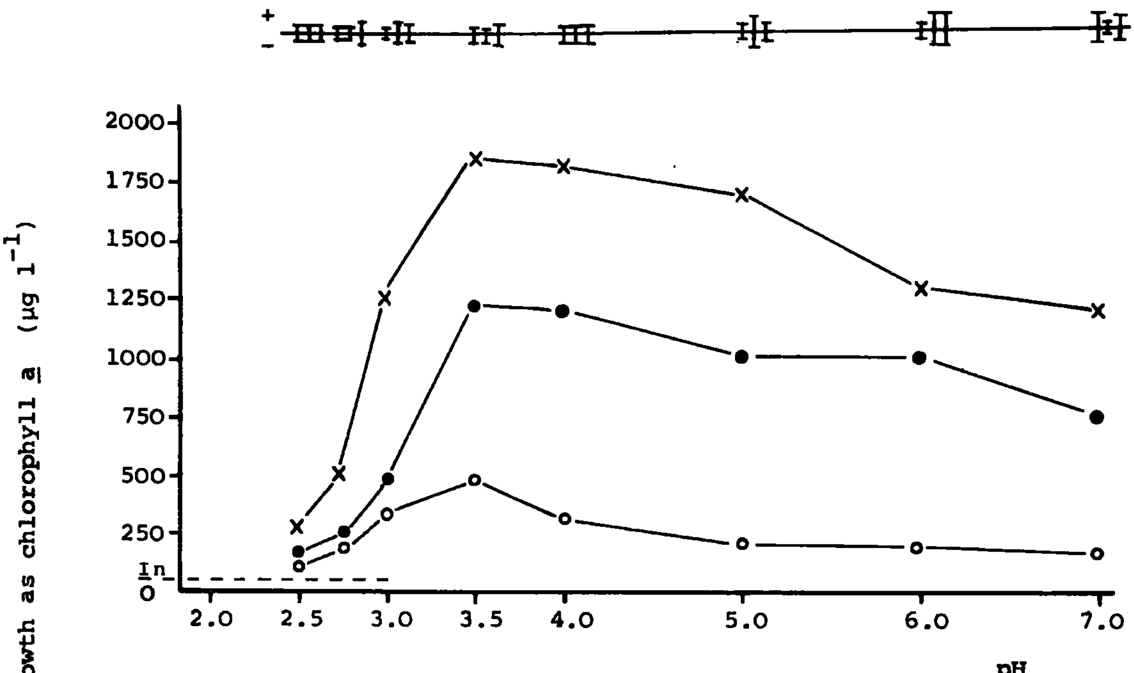
and NaOH, and the growth of _Hormidium rivulare_, at a range of pH values was then examined. The results indicated that the source of H^+ and OH^- ions in the medium did not alter the tolerance of the organism to pH, and therefore as the main source of acid in acid mine drainage was H_2SO_4 this was used for all pH adjustments.

6.23 Effect of varying incubation time on the response of _Hormidium rivulare_ to pH

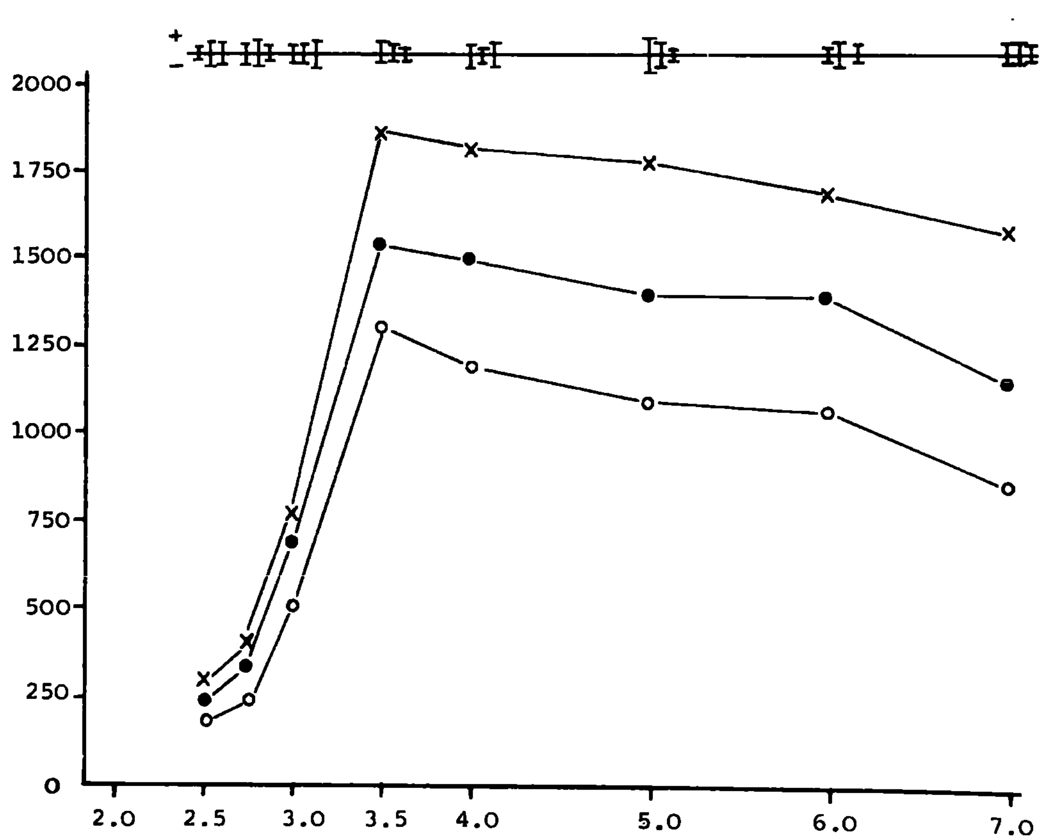
The growth of _H. rivulare_, expressed as µg l^{-1} chlorophyll _a_ was estimated after an incubation time of 4, 7 and 10 days. The results for the range of pH values tested are given in Fig. 6.1, together with the standard errors for each treatment. The results demonstrate that although the amount of growth varied with time, the pattern over the pH values tested was very similar for all three incubation periods. The least difference was found at pH 2.5, where the amount of growth was minimal. However, even at this pH there was some increase with time. As there was little difference in the response to pH over a longer incubation period, all experiments were incubated for 7 days as this proved to be the most convenient period (see 2.83).

6.24 Effect of varying the inoculum size on the response of _Hormidium rivulare_ to pH

The results of this experiment are given in Fig. 6.2. Three sizes of inoculum were tested, 'normal' ½ x normal' and 2 x 'normal'. The normal inoculum was initially based on a visual estimation of the amount of filaments used and was later found to be approximately equal to 40 µg l^{-1} chlorophyll _a_.



o 4 days, • 7 days, x 10 days incubation



• normal inoculum, o ½ normal inoculum, x 2 x normal inoculum

As can be seen from Fig. 6.2, the growth response to pH was not altered significantly by varying the size of inoculum other than by causing either an increase or a decrease in growth compared with the 'normal' inoculum. The standard errors of the individual results indicated that below pH 3.0 there was little difference between the amount of growth produced from the 'normal' and 2 x 'normal' inoculum size.

As a result of this experiment, the 'normal' inoculum size was adopted for the other experiments on _H. rivulare_ because it was felt that over a 7 day period there would be sufficient yield to allow reasonably accurate chlorophyll _a_ estimations.

6.25 Source of inoculum

An experiment was also carried out to determine whether the pH response of _Hormidium rivulare_ altered when freshly isolated material was exposed to a range of pH values as compared with material from the stock cultures. The results indicated that although the stock material had been subcultured routinely for several months at pH 3.0, its response to pH was the same as that of the field material. However, observations showed that when unhealthy or old cultures were exposed to the pH range, the yield was reduced, although the tolerance to pH was very much the same.

6.3 Effect of pH on the growth of acid stream algae

6.31 Growth response to pH in basal media

The influence of pH on the total growth of five species of algae isolated from Brandon Pithouse Acid Stream is shown

Table 6.1 pH values from which species isolated, together with the relative abundance of species in Brandon Pithouse Acid Stream.(Abundance is expressed on 1-5 scale).

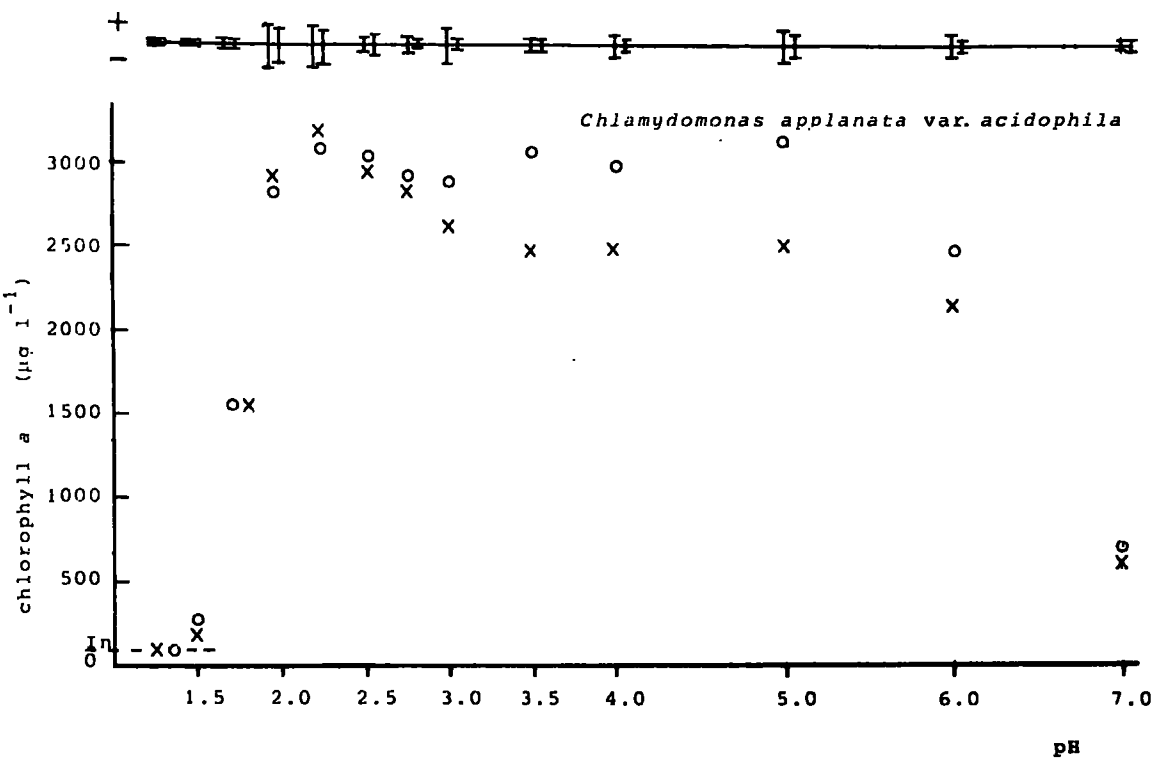
species		estimated relative abundance in stream					
		pH of stream water: 2.6	2.7	2.9	3.0	3.1	3.3
Chlamydomonas applanata var. *acidophila*	2.6	3	3	2	2	2	2
Euglena mutabilis	2.6	5	5	4	3	3	1
Gloeochrysis turfosa	2.6	3	4	3	4	4	3
Hormidium rivulare	3.1	1	1	4	5	5	5
Stichococcus bacillaris	2.6	2	2	1		1	

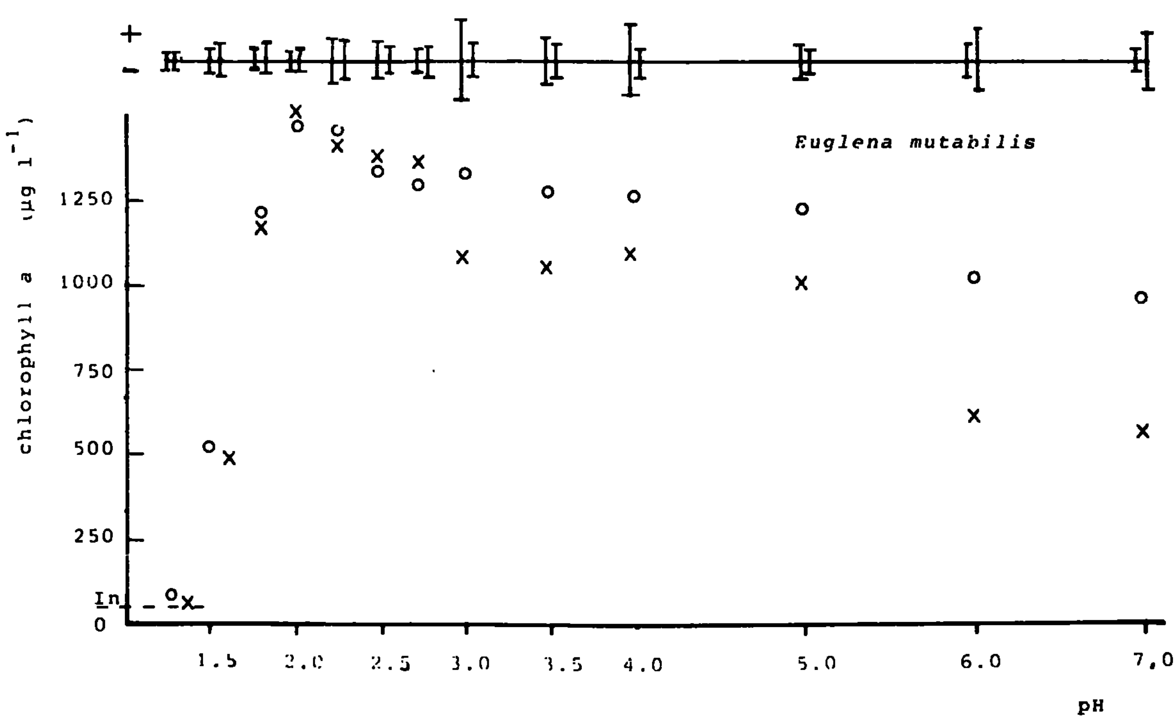
in Fig. 6.3. The pH from which they were isolated and the lowest pH value at which they were recorded in Brandon Pithouse Acid Stream is given in Table 6.1. The results given in this section deal only with the growth response in media without the addition of stream water.

Although all five populations were tolerant to low pH values, there were differences in the response of each organism to the pH range examined. *Euglena mutabilis* and *Chlamydomonas applanata* var. *acidophila* were the most tolerant to low pH, both growing slightly at pH 1.3 and increasing significantly at pH 1.5. The highest yield at the lowest pH was recorded for *Euglena mutabilis*, which produced a total yield of 500 $\mu g\ l^{-1} \pm 75$ of chlorophyll *a* at pH 1.5. Although cells of *Gloeochrysis turfosa* and *Stichococcus bacillaris* survived pH 1.3, the lowest value at which growth occurred was pH 1.5.

The least resistant species to be tested was *Hormidium rivulare*, which did not grow at pH values below pH 2.5, although filaments were found to survive for up to 20 days at pH 2.25.

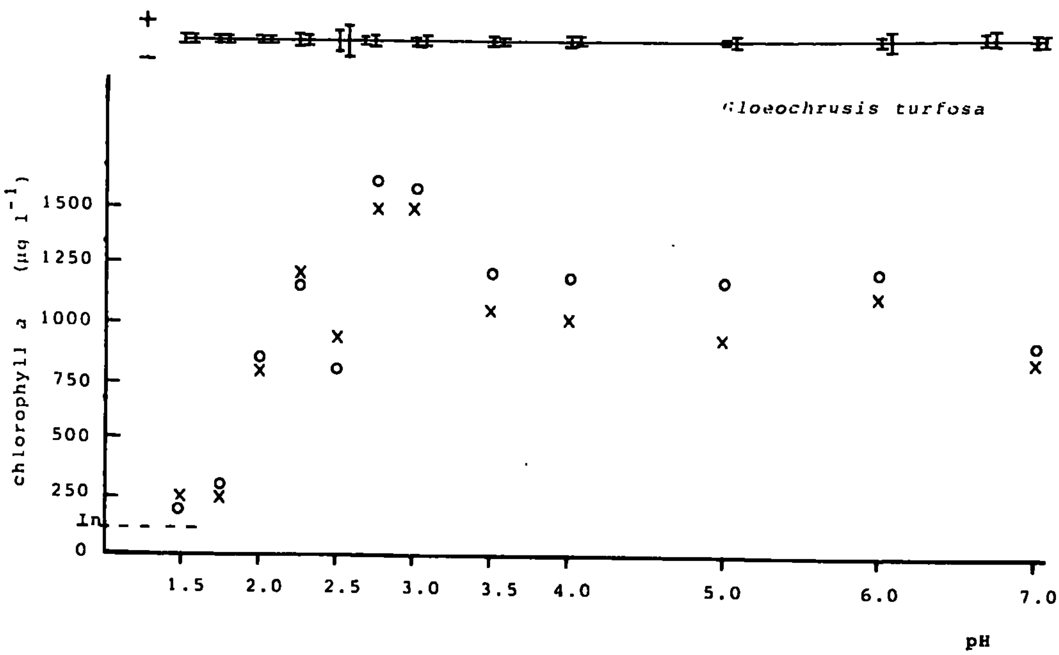
All five populations grew at pH 7.0 even though they were isolated from an acid stream. However, with the exception of *Stichococcus bacillaris*, there was a marked reduction in growth at pH 7.0 compared with that at the optimal growth pH. This was most evident with *Chlamydomonas applanata* var. *acidophila*; this species was also the only species that actually reduced the pH of the medium towards its optimal growth pH range of pH 2.0 to 2.5 (see 7.8).

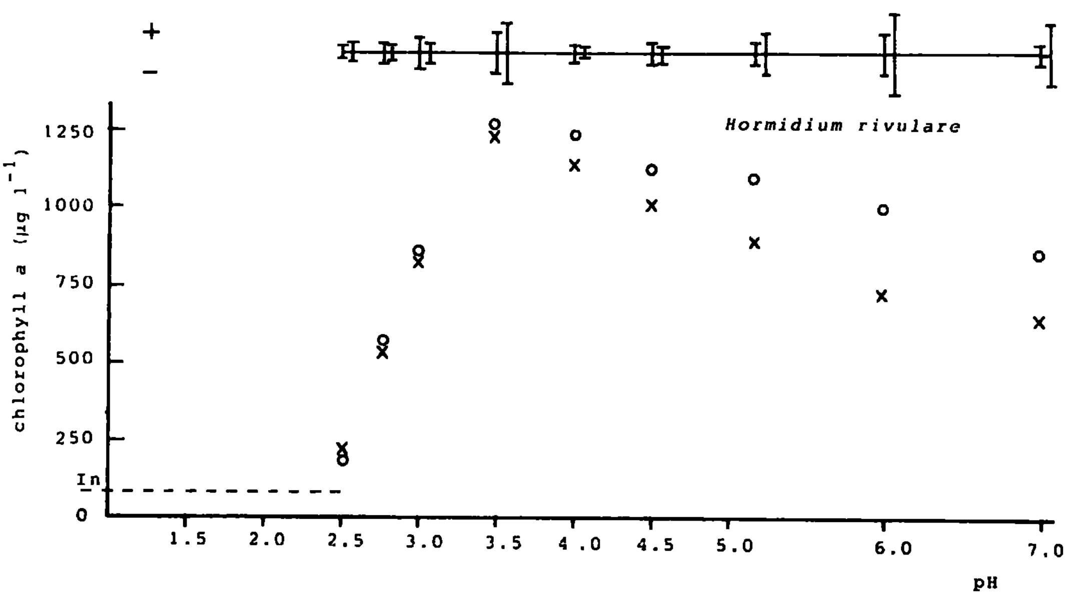


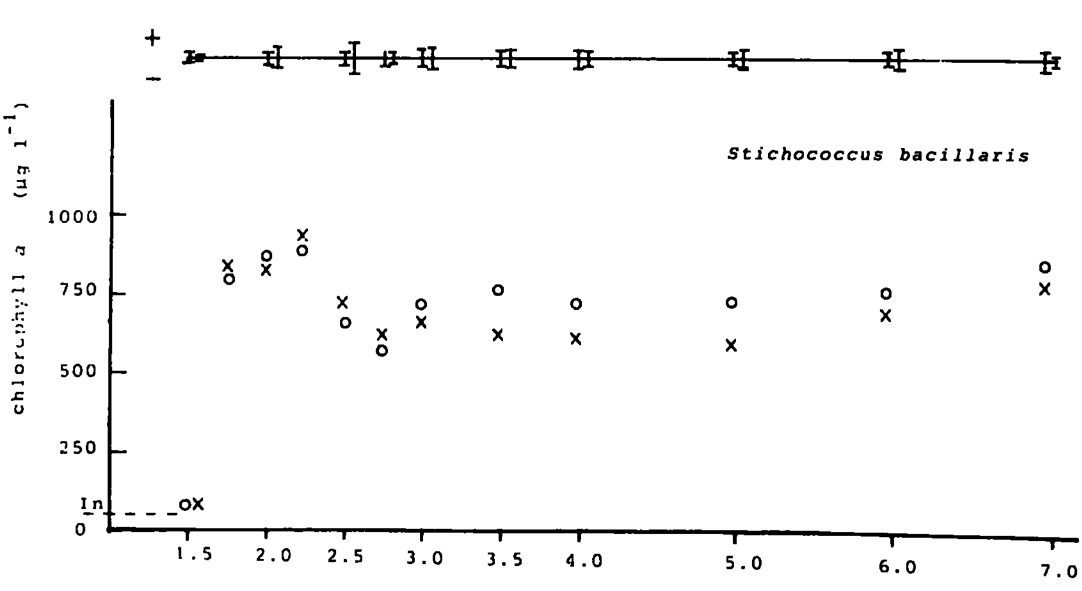


O - medium without stream water

X - medium with 10% stream water


+
-
Gloeochrusis turfosa
chlorophyll a (µg l-1)
1500
1250
1000
750
500
250
In
0
1.5
2.0
2.5
3.0
3.5
4.0
5.0
6.0
7.0
pH


+
-
Hormidium rivulare
chlorophyll a (µg l-1)
1250
1000
750
500
250
In
0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
6.0
7.0
pH


+
-
Stichococcus bacillaris
chlorophyll a (µg l-1)
1000
750
500
250
In
0
1.5
2.0
2.5
3.0
3.5
4.0
5.0
6.0
7.0
pH

6.32 Effect of the addition of filtered acid stream water on the pH growth response

In order to simulate field conditions where precipitation of much of the dissolved iron occurs at pH 3.0 - 3.5, experiments were carried out on the five test populations, with the medium modified to include 10% membrane filtered stream water. Table 6.2 includes the analysis of the medium after the pH had been raised above pH 3.0. These results demonstrate that the precipitate which formed in the medium consisted mainly of Fe and Al, although small amounts of several other ions were also removed from solution. However, even after precipitation, the concentrations of the ions which decreased were still greater than those in the control medium.

As can be seen from Fig. 6.3 the presence of stream water had little effect on growth at the lower pH values, but at higher values (pH 3.0), reduced growth occurred in the presence of the stream water. This was most noticeable with *Chlamydomonas applanata* var. *acidophila*, *Euglena mutabilis* and *Hormidium rivulare* and least obvious with *Stichococcus bacillaris*.

6.33 Effect of prolonged growth at pH 6.0 on the pH tolerance of *Euglena mutabilis* and *Hormidium rivulare*

As mentioned in 2.83, the influence of prolonged growth at pH 6.0 on the pH tolerance of these two populations was compared with that of freshly isolated material. The stock cultures were maintained at pH 6.0 for two months and then subjected to the full pH range used in previous experiments. In both instances, the growth response was identical to that

Table 6.2

Comparison of the concentrations of ions in Chu 10 + 10% acid stream water at pH 2.5 and pH 4.5. Concentrations as mg l^{-1}.

	Chu 10 + 10% acid stream water pH 2.5	Chu 10 + 10% acid stream water pH 4.5	difference pH 2.5 - pH 4.5
Na	7.4	7.3	0.1
K	2.5	2.5	
Mg	11.3	11.2	0.1
Ca	15.2	15.2	
Zn	0.51	0.37	0.14
Cu	0.096	0.090	0.006
Mn	0.80	0.79	0.01
Fe	13.1	1.40	11.7
Al	7.8	2.65	5.2
Pb	0.005	0.003	0.002
Co	0.07	0.07	
Ni	0.10	0.10	
PO_4-P	2.3	2.1	0.2
NH_4-N	8.5	8.4	0.1
NO_3-N	7.2	7.0	0.2
SO_4-S	38.0	33.4	4.6
Si	6.5	5.0	1.5

shown in Figs. 6.2 and 6.3.

A similar experiment was also conducted to see whether Hormidium rivulare could be induced to grow at pH values lower than 2.5. In this experiment the test population was freshly isolated from reach 1 of Brandon Pithouse Acid Stream at pH 2.65 where it had been growing for one month. The results did not show any increase in tolerance of the organism to pH and a similar growth response to pH was obtained as that shown in Fig. 6.3.

6.34 Comparison of the pH limits for growth in the field with those in the laboratory

The pH tolerances of the five species in the laboratory are compared with the tolerances observed in Brandon Pithouse Acid Stream and also the lowest value at which they were recorded elsewhere in England. These data are given in Table 6.3. It can be seen that all five species could grow in the laboratory at lower pH values than in the stream from which they were isolated. There was a similarity between the laboratory lower limits and those found under field conditions. The lower pH limit of 2.5, found for Hormidium rivulare in the laboratory, corresponds to the lower limit in the field. All other populations were found to grow at slightly lower pH values in the artificial medium than recorded in the field. However, as only one reach was recorded at pH 1.5 and none at pH 1.3, it is possible that these species could grow at lower values in the field.

Table 6.3 Comparison of lower pH limits in the field and in laboratory

	Chlamydomonas applanata var. *acidophila*	*Euglena mutablis*	*Gloeochrysis turfosa*	*Hormidium rivulare*	*Stichococcus bacillaris*
lowest pH at which detectable growth	1.3	1.3	1.5	2.5	1.5
lowest pH at which cell survival, but not growth occurred			1.3	2.25	1.3
lowest pH at which found in stream from which isolated	2.6	2.6	2.6	2.6	2.6
lowest pH at which species recorded anywhere in England	1.8	1.5	1.8	2.5	1.8

6.35 Effect of pH on morphology

Morphological differences were apparent near the lower limits in populations of all five species, although these differences were not always detectable in every cell. The morphological differences and the pH range at which they occurred are summarized in Table 6.4.

Stichococcus bacillaris showed the most obvious variations, whilst cells of *Euglena mutabilis* were relatively uniform in size and shape over the pH range tested. With *Stichococcus bacillaris* these differences were apparent at pH values which had little effect on growth rate, whereas with the other species, obvious morphological changes were associated with a marked reduction in growth rate.

Similar morphological effects to those summarized in Table 6.4 were also observed in cultures where the acid stream water was added. However, at pH values above 3.0 brown iron materials were often attached to the mucilage of *Gloeochrysis turfosa*, *Hormidium rivulare* and *Stichococcus bacillaris*. Apart from those mentioned above, no other abnormalities appeared to be caused by the presence of the precipitate.

The variations in morphology of these species is given in more detail in chapter 7.

6.4 Effect of heavy metal concentration on *Hormidium rivulare* and *Euglena mutabilis* over a range of pH values

As most acid mine drainage waters were found to contain large concentrations of heavy metals (see 4.13), experiments were carried out into the effects of Zn on the growth of

Table 6.4 Influence of pH on morphology

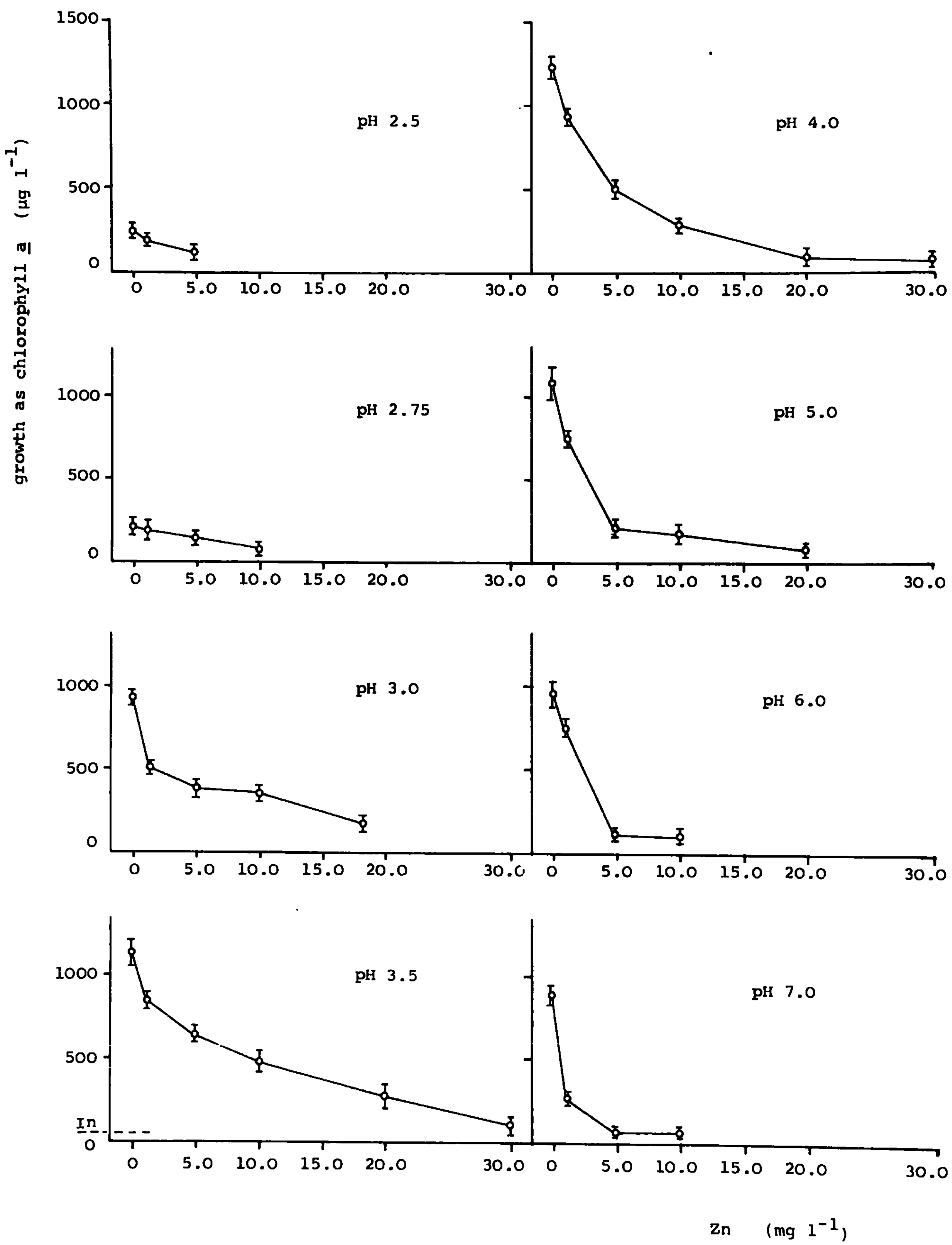
Chlamydomonas applanata var. acidophila	
1.3 - 1.5	smaller cells, thicker walls, fewer motile cells than at higher pH values
Euglena mutabilis	
1.3 - 1.5	more non-motile, rounded cells than at higher pH values; occurrence of some "monster" cells (i.e. cells that do not divide again and are considerably larger than the average cell.
Gloeochrysis turfosa	
1.5 - 2.25	cells relatively small (mostly with diameter of 3.5 μm)
2.5 +	cells larger, mostly reaching a diameter of 6-7 μm before division, characteristic dimensions of the species.
Hormidium rivulare	
2.5 - 2.75	twisted filaments, accompanied by swollen or otherwise anomalous shaped cells; more geniculations; chloroplasts smaller and an uncharacteristic yellow-green.
2.75-3.0	mucilage production especially obvious.
Stichococcus bacillaris	
1.5 - 1.75	many cells contorted to 'S' or 'U' shapes, in spite of near optimal growth-rate; no chains, but many clumps of cells.
2.0 - 2.5	long chains of cells present (up to 40 cells per chain), these chains being unusually stable mechanically, probably as a result of the larger amount of mucilate produced; a few 'contorted' cells present.
2.75+	all cells rod-shaped; chains being fewer and shorter with increase in pH.

Hormidium rivulare and *Euglena mutabilis*. An additional experiment was also conducted on the influence of Cu on the growth of *H. rivulare* at a range of pH values. These two species were chosen because they represented the most, and the least, pH tolerant organisms cultured.

6.41 Effect of Zn concentration on *Hormidium rivulare*

The influence of a range of Zn concentrations, from 0 to 30.0 mg l^{-1}, over a pH range of 2.5 to 7.0, on the growth of *H. rivulare* is given in Fig. 6.4. The overall effect of increased Zn levels in the medium was to cause a decrease in growth of all pH values tested. However, there was considerable variation in the degree of tolerance of the alga to Zn at different pH values. At pH 2.5 it was the most sensitive to Zn, being effectively killed by concentrations above 5.0 mg l^{-1} Zn. Whilst at pH 3.5 and 4.0, the tolerance was considerably increased and growth was recorded at 30 mg l^{-1} Zn. Above pH 4.0 the decrease in growth rate at 0 mg l^{-1} Zn was accompanied by a decrease in tolerance to Zn. At pH 7.0 there was only a slight increase in growth recorded at 5.0 and 10.0 mg l^{-1} Zn. Whilst the organism appears to be more tolerant at pH 7.0 than pH 2.5, the decrease in growth at pH 7.0 in the presence of 5.0 mg l^{-1} Zn, was greater when compared with growth at pH 7.0 and no Zn which was greater also than the growth rate at corresponding levels of Zn at pH 2.5.

This implied that Zn was more toxic at pH 6.0 to pH 7.0 than at pH 2.5. Comparison of the results in Fig. 6.4 with the field levels recorded for Brandon Pithouse Acid Stream, reach



growth as chlorophyll a (µg l⁻¹)
1500
1000
500
0
pH 2.5
pH 4.0
pH 2.75
pH 5.0
pH 3.0
pH 6.0
pH 3.5
pH 7.0
In
0
5.0
10.0
15.0
20.0
30.0
Zn (mg l⁻¹)

10 (Zn 1.0 mg l^{-1}) show that the population was considerably more tolerant to Zn at all pH values than the levels to which it was exposed in the field.

6.42 Effect of Zn on <u>Euglena mutabilis</u>

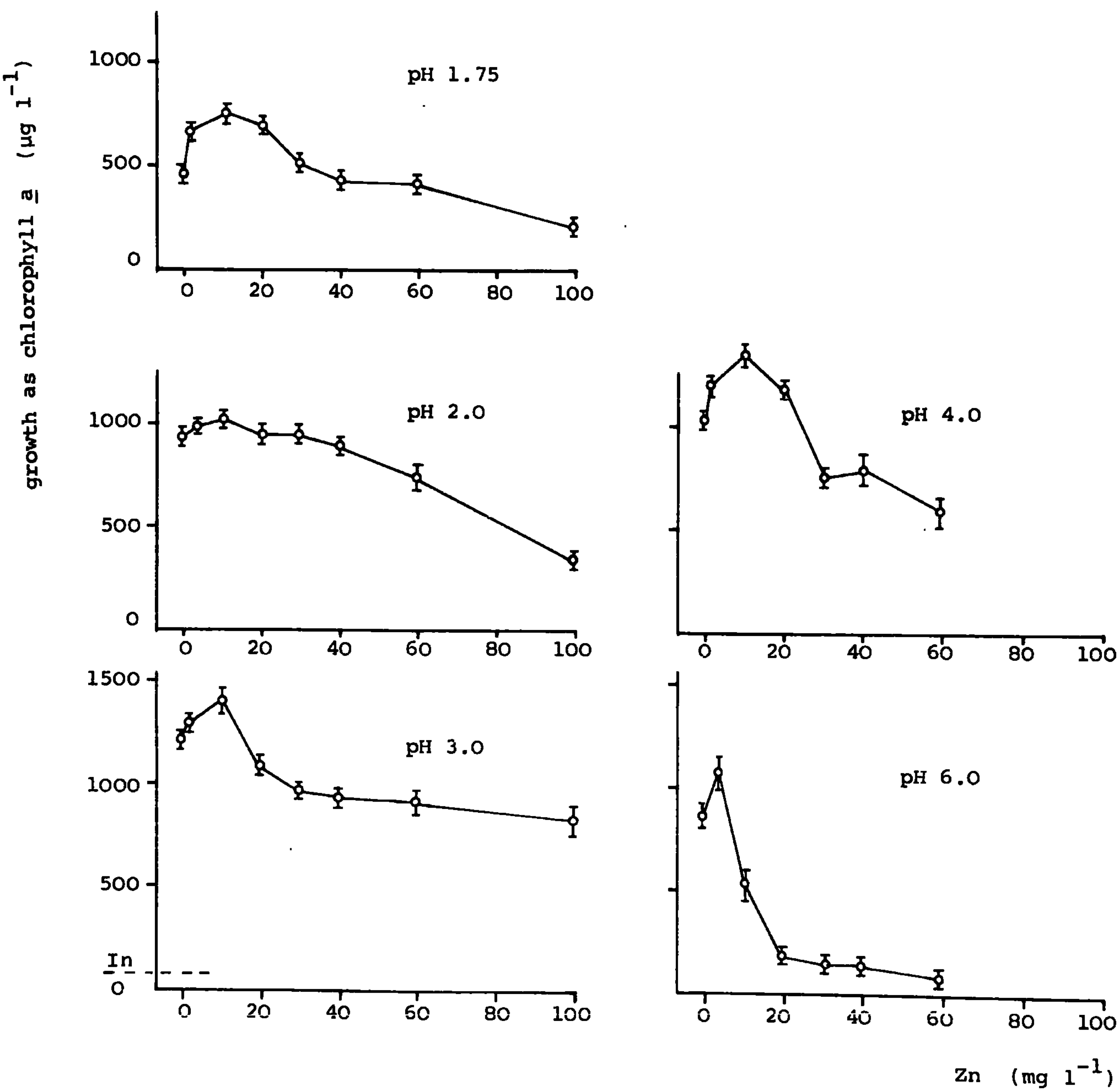
The tolerance of <u>Euglena mutabilis</u> to a range of Zn concentrations (0 - 100 mg l^{-1}), over a range of pH values (1.75 - 6.0) is shown in Fig. 6.5. The effect of increasing the levels of Zn was to cause a decrease in the growth rate at all pH values. As was found for <u>Hormidium rivulare</u>, there was considerable variation in the degree of tolerance to Zn .. There was greater tolerance to Zn at the lower pH values than at the higher values tested. At pH 1.75 there was better growth at 100 mg l^{-1} Zn than there was at 60 mg l^{-1} Zn at pH 6.0. The greatest tolerance to Zn and the maximum growth occurred at pH 3.0; whilst at pH 6.0 the organism showed the least resistance to Zn , growing poorly at concentrations greater than 10 mg l^{-1}.

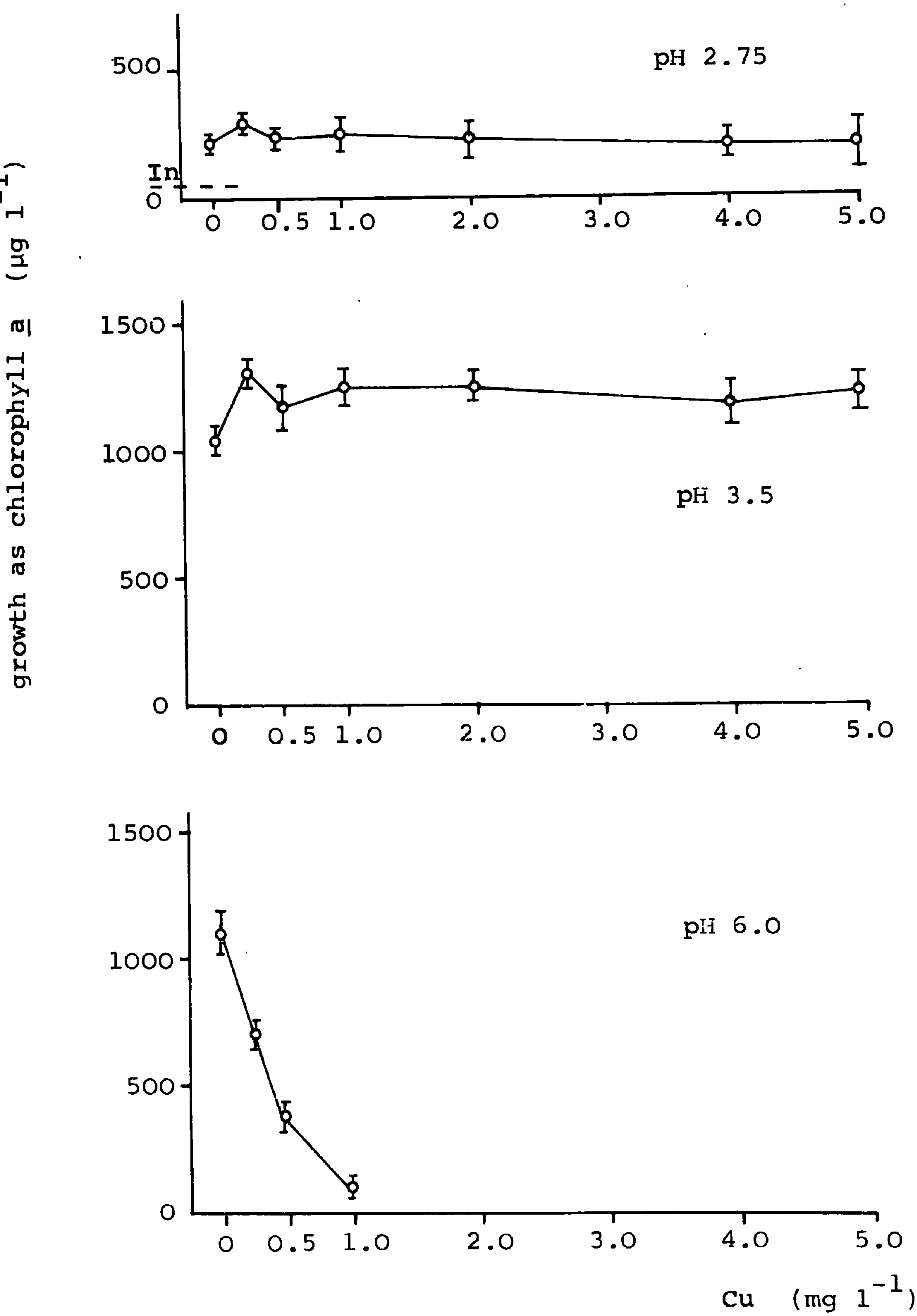
Besides being more tolerant to Zn than the test population of <u>H. rivulare</u>, there was also some evidence to suggest that <u>E. mutabilis</u> had a requirement for quite large concentrations of the element. This was shown by the increasedgrowth at all pH values in the presence of between 1.0 and 10 mg l^{-1} Zn .

6.43 Effect of Cu on the growth of <u>Hormidium rivulare</u>

The effect of Cu on the growth of <u>Hormidium rivulare</u> at pH 2.75, 3.5 and 6.0 is given in Fig. 6.6. The results show that the population was resistant to much larger concentrations

Fig. 6.5 Effect of Zn concentration on the total growth of Euglena mutabilis over a pH range. Vertical bars represent the standard error of the mean of 4 replicates.




pH 2.75
pH 3.5
pH 6.0
growth as chlorophyll a (μg l^{-1})
Cu (mg l^{-1})
In
500
1500
1000
0
0.5
1.0
2.0
3.0
4.0
5.0

of Cu than it had previously encountered in Brandon Pithouse Acid Stream (Cu - 0.7 mg l^{-1}). There was also a marked increase in toxicity of Cu with an increase in pH. The greatest resistance to Cu was at the optimum growth pH, however, unlike the response to Zn Hormidium rivulare tolerated the same range of Cu (0 - 5.0 mg l^{-1}) at pH 2.75 as it did at pH 3.5, except that the growth was considerably greater at pH 3.5. At pH 6.0 there was much less resistance to Cu, with a marked decrease in total growth as the level increased from 0 to 1.0 mg l^{-1}.

The growth curves at pH 3.5 and 2.75 suggest that 5.0 mg l^{-1} Cu had little effect on growth. (Unfortunately, further tests were not carried out to determine the limiting concentration

6.5 pH tolerance of populations of *Hormidium* spp. not previously exposed to low pH conditions

6.51 *Hormidium rivulare*

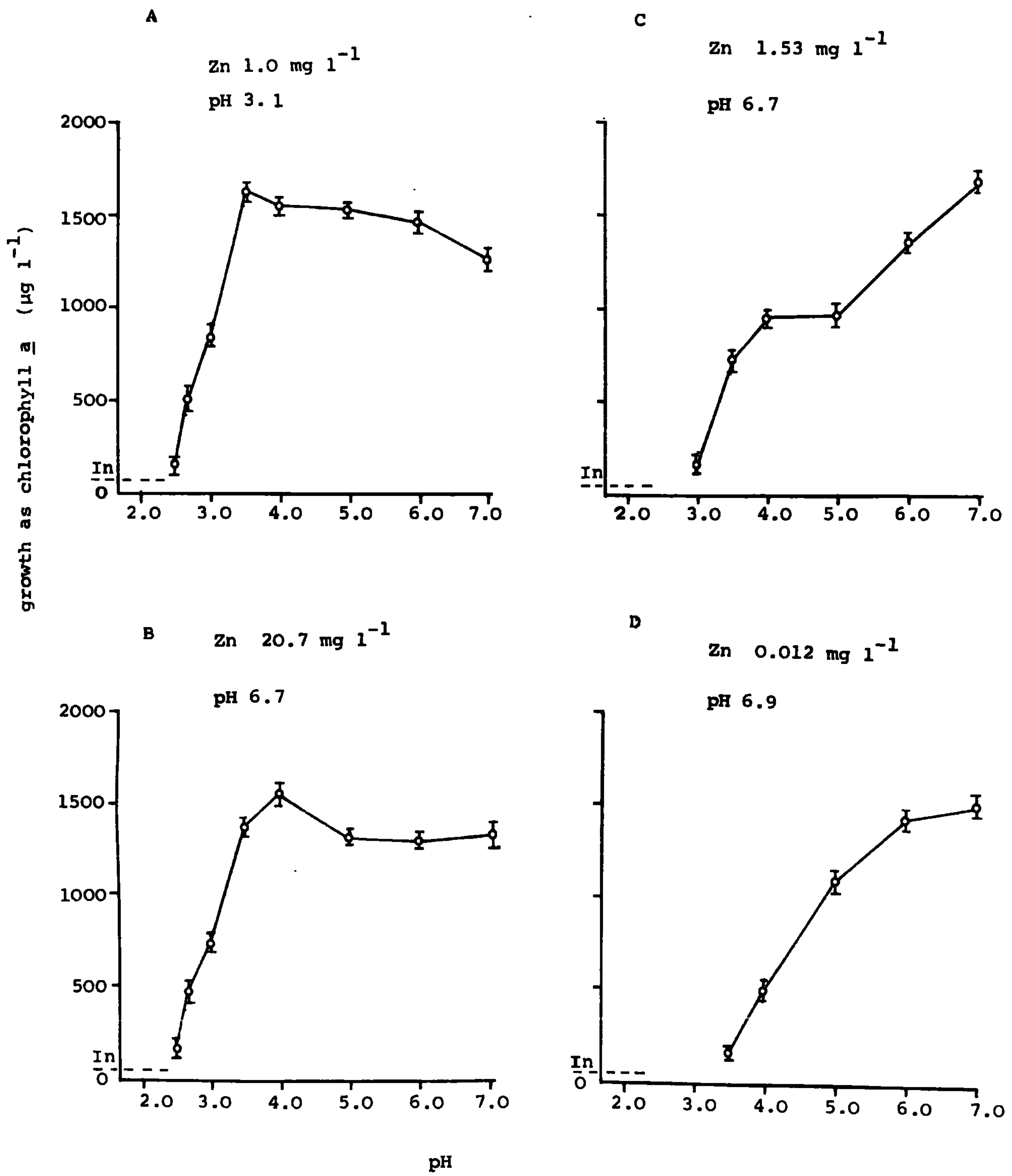
As the population of *H. rivulare* isolated from Brandon Pithouse Acid Stream at pH 3.1 had shown pre-adaptation to large concentrations of heavy metals, experiments were carried out to determine the growth response of non pH adapted populations to a range of pH values.

For the purpose of these experiments, populations were isolated from non acid sites. The pH and other chemical parameters measured for the water from which they were isolated, are given in Table 6.5 for the three different populations tested. The growth of the three populations was tested over the usual pH range and the total yield measured after 7

Table 6.5 Details of sites and water chemistries (mean values) from which *Hormidium rivulare* populations were isolated. (Information for B, C and D from P. J. Say)

population	A	B	C	D
name of stream	'Brandon Pithouse Acid Stream A'	'Old Mine Gill'	Gillgill Burn	'Cookshold Stream'
U.K. grid ref.	NZ 217410	NY 795440	NY 783437	NZ 339427
Durham stream reach code	0127/10	0104/01	0093/85	0151/05
pH	3.1	6.7	6.7	6.9
Mg	82	4.1	3.7	75.1
Ca	98	17.1	14.3	117
Zn	1.0	20.7	1.53	0.012
Cu	0.24	0.004	0.002	0.006

Fig. 6.7 pH growth response of four populations of Hormidium rivulare. Vertical bars represent the standard error of the mean of 4 replicates.



days incubation. The results are given in Fig. 6.7 (A to D). They demonstrate that while the populations B, C and D grew at lower pH values than they had previously encountered, the response to pH was different for each population. Populations C and D (Fig. 6.7) were the least resistant to low pH and did not grow at pH values of less than pH 3.5. Their maximum growth occurred at pH 7.0, whereas population B (Fig. 6.12b) showed a similar response to pH as was recorded for the acid stream Hormidium rivulare (population A) isolated from pH 3.1. As shown in Table 6.5, the main differences in the water chemistry of the three non acid sites was the concentration of Zn . The results indicated a relationship between the concentration of Zn in the stream water and the organisms tolerance to pH. The least pH tolerant population (population D) was also the population which had been exposed to the lowest Zn concentration, and the most pH tolerant population besides the acid stream population, was that isolated from water containing 20 mg l^{-1} Zn.

6.52 Hormidium flaccidum and H. scopulinum

The influence of pH on the growth of these two species was tested to determine whether other Hormidium spp. responded to pH in a similar manner as H. rivulare. The growth of two different populations isolated from non acidic streams, but bearing Zn concentrations of 1.21 mg l^{-1} and 1.0 to 2.0 mg l^{-1}, were exposed to a pH range from 2.5 to 7.0. As can be seen from Table 6.6, the two species varied in their tolerance to pH, and were also less tolerant than H. rivulare. Neither

Table 6.6

The influence of pH on the growth of *Hormidium flaccidum* and *H. scopulinum* (growth as chlorophylla ug l^{-1})

pH	*H. flaccidum*	*H. scopulinum*
3.5	no growth	no growth
4.0	100	150
4.5	280	380
5.0	650	950
6.0	1000	1100
7.0	1200	1100

species was as resistant to pH as the least resistant population (D) of H. rivulare. Maximum growth was recorded at pH 7.0 in both cases, and although both species were still viable at pH 4.0 after 7 days, growth was poor below pH 5.0.

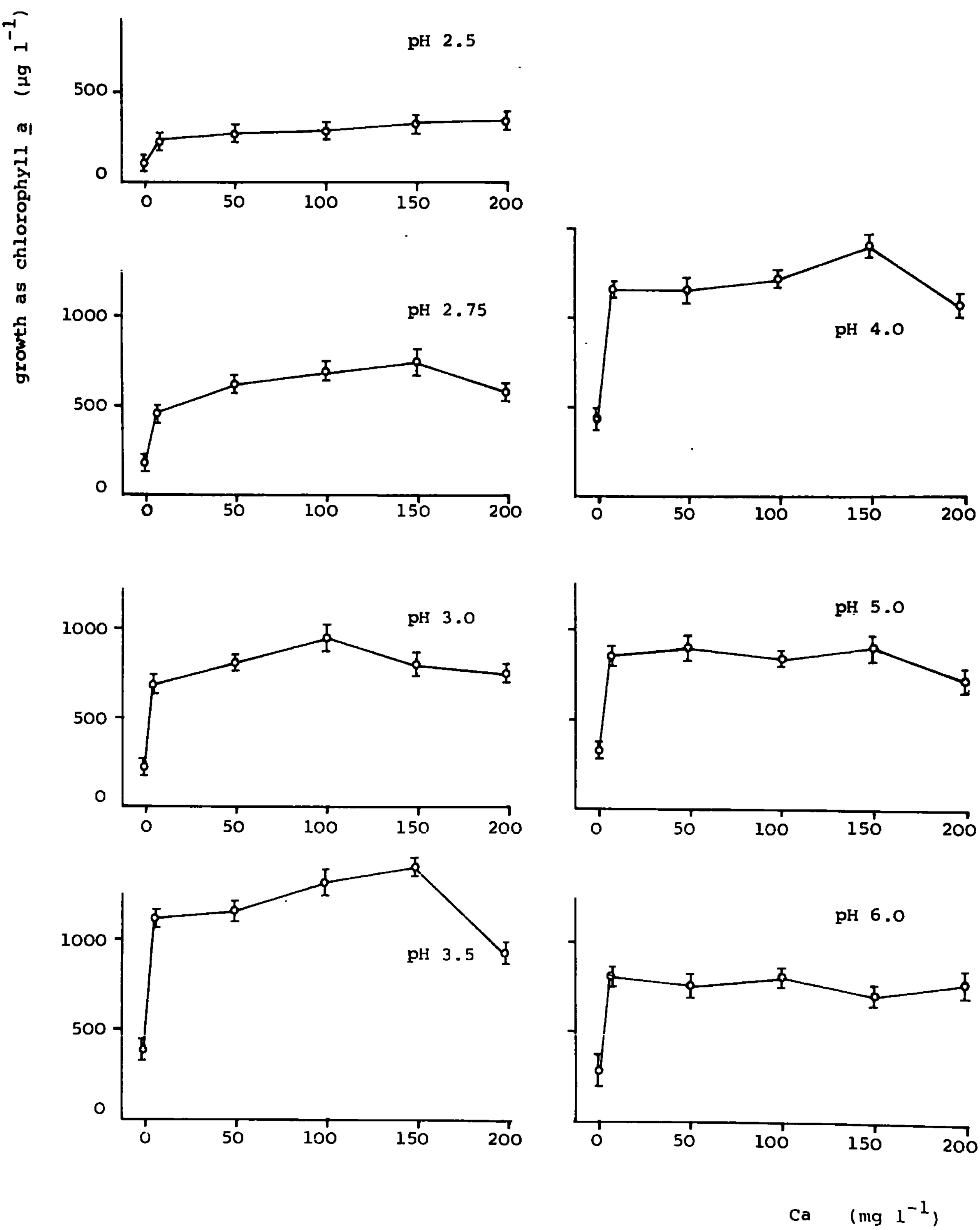
6.6 Effect of Ca, Mg, PO_4-P and Al on the response of Hormidium rivulare to pH.

It has been shown that Ca, Mg and PO_4-P all antagonize the toxicity of Zn to a Zn -resistant populations of Hormidium rivulare isolated from an alkaline stream and grown under non acid conditions (Say & Whitton, in press). As the experiments described in 6.5 suggested that populations from Zn rich environments were 'pre-adapted' to low pH, experiments were carried out on the acid stream populations to see whether the elements mentioned above would act similarly in increasing tolerance to low pH. In addition, the influence of varying concentrations of aluminium was also tested, as this element invariably occurred in large concentrations in the acid streams. The effect of increasing the levels of Fe and SO_4-5 in the medium have already been briefly reported in 6.32 and 2.82 and were not found to improve growth at low pH.

6.61 Ca

The effect of increasing the concentration of Ca in the medium from 0 to 200 mg l^{-1}, over a pH range of 2.5 to 6.0 is given in Fig. 6.8. It can be seen that raising the level of Ca above the minimum level tested (10 mg l^{-1}0), brought about only a slight improvement in growth at the lower

Fig. 6.8 Effect of Ca on the total growth of <u>Hormidium rivulare</u> over a pH range.



pH values and none at the higher pH values. At all pH values the minimum growth occurred where no Ca was included, thus demonstrating the organisms requirement for that element. For all pH values, except pH 2.5 and 6.0, the amount of growth decreased in the presence of 200 mg l^{-1} Ca. This was most marked at pH 3.5 and 4.0.

6.62 Mg, PO_4-P, Al

A range of concentrations of Mg, PO_4-P and Al were tested at pH 2.75 and pH 3.5, and the results are given in Fig. 6.9. It can be seen that an increase in Mg and PO_4-P concentrations did not improve growth at either pH value tested. The most marked increased in growth at pH 2.75 occurred with an increase in Al concentration. Growth at both pH values was considerably reduced when all three elements were absent from the media, demonstrating a requirement for quite large concentrations of these ions. As found for Ca, growth was inhibited by the largest concentration of all three elements tested at pH 3.5, although the decrease was only slight with PO_4-P. Similar inhibition was not demonstrated at pH 2.75 for all three ions.

6.63 Effect of Ca on the toxicity of Zn to Hormidium rivulare

As the addition of the elements mentioned in the previous sections did not cause a marked improvement in growth at low pH, an experiment was conducted to determine the influence of Ca on the toxicity of Zn. The results given in Fig. 6/10 demonstrate clearly that the toxicity of Zn was decreased by the presence of Ca at all pH values.

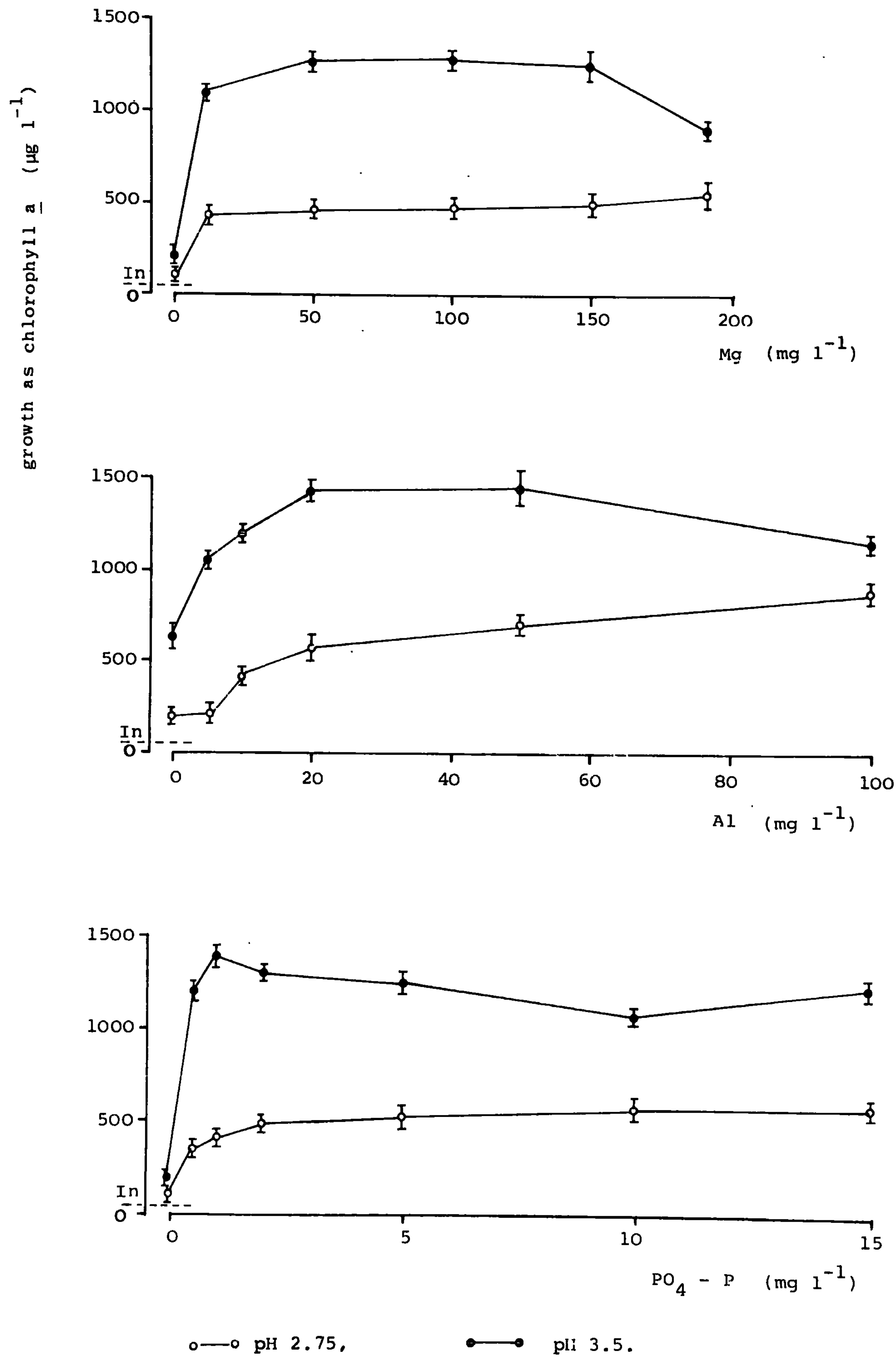
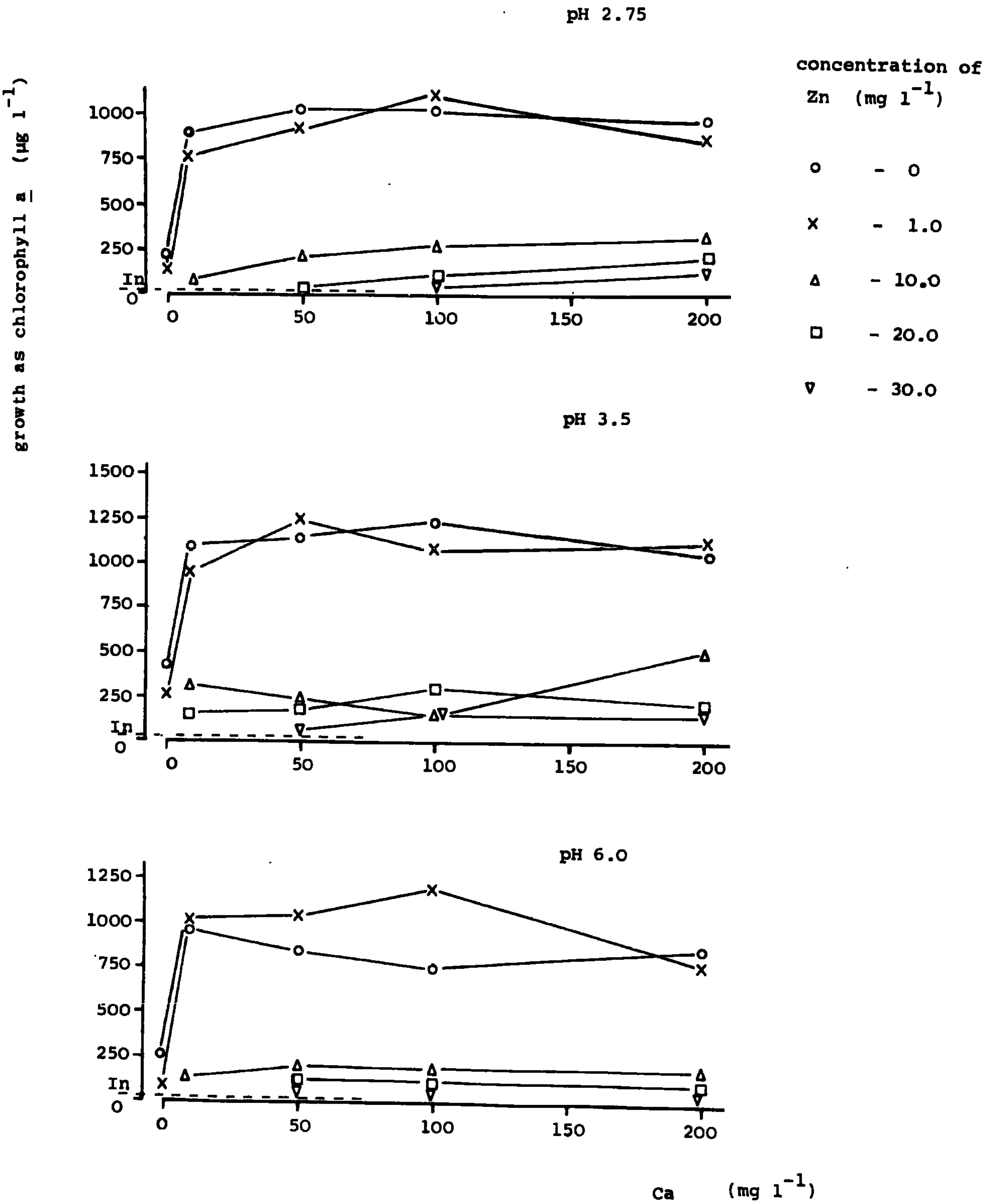

growth as chlorophyll a (μg l^{-1})
1500
1000
500
In
0
0
50
100
150
200
Mg (mg l^{-1})
1500
1000
500
In
0
0
20
40
60
80
100
Al (mg l^{-1})
1500
1000
500
In
0
0
5
10
15
PO$_4$ - P (mg l^{-1})
o—o pH 2.75,
●—● pH 3.5.

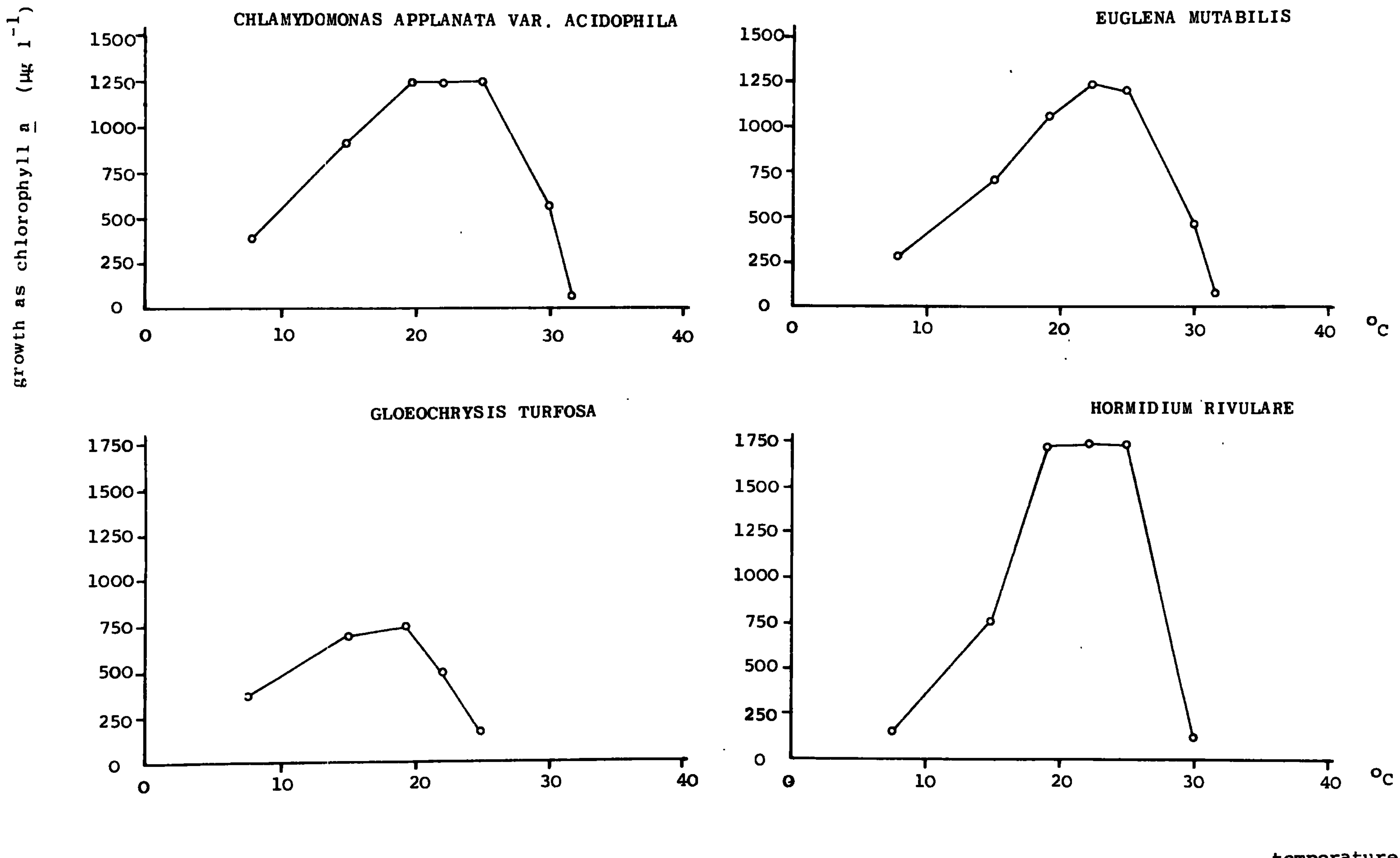
Fig. 6/ 10 Effect of Ca on the toxicity of Zn to Hormidium rivulare over a pH range. Values given are the mean of 2 replicates.



For example, at pH 2.75 and 6.0, growth was recorded in the presence of 30 mg l^{-1} Zn when concentrations of Ca were between 100 and 200 mg l^{-1}; whereas at 10 mg l^{-1} Ca, it could only tolerate 10 mg l^{-1} Zn. The greatest tolerance to Zn, at the lowest Ca level, occurred at the optimum growth pH of 3.5. The response to Ca was similar to that observed at higher pH conditions with other populations of Hormidium rivulare (Say & Whitton, in press).

6.7 Effect of temperature on growth of four acid tolerant species

The possibility was tested that species which can tolerate acidic conditions may also have the ability to grow at another environmental extreme, for instance, high temperature. The species used were: Chlamydomonas applanata var. acidophila, Euglena mutabilis, Gloeochrysis turfosa and Hormidium rivulare. Each species was grown in media at a pH value within it's individual optimum pH growth range and incubated at temperatures ranging from 8°C to 40°C. The results given in Fig. 6/11 show that none of the acid tolerant species were capable of growth at temperatures above 35° C. The most resistant species were Chlamydomonas applanata and Euglena mutabilis, which grew at 32°C. However, at the maximum temperature the growth rate was very much reduced compared with that at 25°C. The chrysophyte was the most temperature sensitive species tested, and did not grow above 25°C. All four species grew at a greatly reduced rate at the lower temperatures and Hormidium rivulare was the


growth as chlorophyll a (µg l⁻¹)
CHLAMYDOMONAS APPLANATA VAR. ACIDOPHILA
EUGLENA MUTABILIS
GLOEOCHRYSIS TURFOSA
HORMIDIUM RIVULARE
1500
1250
1000
750
500
250
0
1750
0
10
20
30
40
°C
temperature

most affected. This supports the field observation that *H. rivulare* was much less common in winter than in summer (5.9 and 7/10).

7. MORPHOLOGY AND ECOLOGY OF SPECIES RECORDED AT pH 3.0

7.1 Introduction

Many of the species recorded during the surveys of acid streams were found to be morphologically variable, both between populations and within the same population. It was felt, therefore, that it would be of value to comment on the morphology of the individual species where they were markedly different from descriptions given in the literature. In addition, some data are also given for the species on their distribution, frequency of occurrence and, where possible, environmental factors which were thought to have some influence on their presence or absence. Where relevant, reference to the laboratory experiments (see Chapter 6) have also been included. In cases where a species was recorded on only one or two occasions it was only possible to give brief accounts. Where it was not possible to give binomials to a presumed species, descriptions are given in more detail, apart from those cases mentioned above.

For ease of reference, some comparisons with the literature and some discussion of the individual species have been made in this section. The algal species have been considered in order of their frequency, according to Table 4.4. The mosses and flowering plants have been included at the end of the chapter. Because of the individual treatment of the species in this chapter, each one has been dealt with in five sections. These are: I. Morphology and taxonomic observations: II. Distribution and frequency of occurrence: III. Habitat: IV. Factors influencing the occurrence of species: V. Comparison with available literature. Where it was considered relevant, drawings of some species have been included. In cases where no data were available for a particular section, the relevant section has been omitted.

7.2 *Euglena mutabilis*

I. Morphology was found to be reasonably uniform in all populations examined. The cell length tended to be smaller than expected for this species, ranging between 60 µm and 120 µm, with the majority of cells recorded falling between 70 µm and 90 µm.

The occurrence of large 'monster' cells (cells which are thought not to divide again) and rounded, non motile cells, increased at pH conditions of less than pH 2.0. This was also noted in cultures growing at pH 1.3 - 1.75 (see 6.35).

In several populations it was found that there was a marked increase in paramylon and lipid bodies. These appeared to be in greatest abundance in conditions in which the growth rate was reduced, or where high concentrations of nutrients occurred. In such populations the bodies were generally associated with larger and therefore probably older cells. Similar observations were made in cultures grown in nutrient rich media.

II. As previously mentioned (see 4.41), the alga was found not only to be the species most frequently and widely occurring, but also the one most abundant below pH 3.0 (see Tables 4.7 and 4.8). It was not unusual to find as much as 80% of the stream bed (see 4.44) covered with a film of this alga several cells thick.

Although no estimation was made of its productivity, relatively large areas (several square cm) were covered by the organism and a standing crop of 2.5 $\mu g\ mm^{-2}$ chlorophyll a (see 5.82) was recorded on several occasions from site 3 and site 16. A characteristic of this species was its ability to rapidly colonize new acid habitats (eg. site 10). This would suggest that the species was geographically widespread and a common member of most aquatic habitats.

There did not appear to be any marked seasonal variation in occurrence of this species although the estimated relative abundance data from Brandon Acid Stream (Fig. 5.14) indicated that there was a decrease in abundance during the late winter, with maximum growth occurring in late spring and early summer.

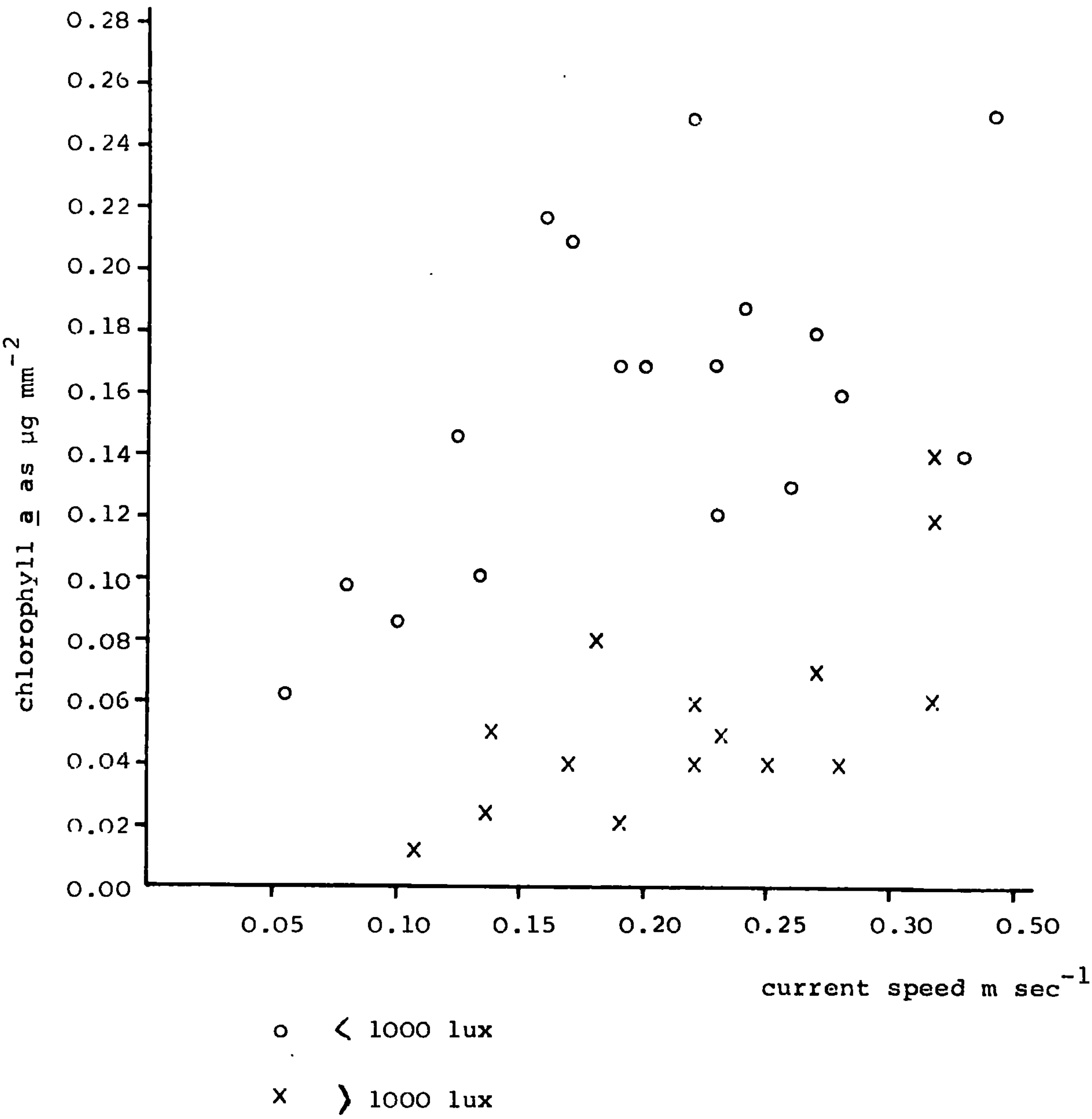
III. Although E. mutabilis was recorded in all types of habitat investigated in this study, there appeared to be some indication of habitat preferences. The organism was found to grow in greatest density in streams with a stable but penetrable substratum and a reasonably consistent medium current speed (estimated to be about 0.3 $m\ sec^{-1}$). In streams where the substratum was soft and friable, as was commonly found in reaches around pH 3.0 where iron oxide was continuously precipitating out and the current speed was slow, then growth tended to be reduced. This was possibly due to a continuously changing substratum which prevents the usual formation of a film of cells. Likewise, it was unusual to find dense growth on bedrock, sheet clay, or compacted iron oxide precipitate or when current speeds were fast (approximately 0.4 m Sec^{-1}). In these conditions growth tended to be at the edge of the stream in the slower currents.

The organism also appeared to have a preference for situations which did not receive direct sunlight. In exposed reaches the greatest density was often found in shaded areas, such as the north facing bank of the stream. The effect of light is dealt with further in section IV. IV. Good growth was reported in the most extreme conditions of low pH (site 4, reach 5; pH 1.5) highest acidity (site 13, reach 2; 64000 $CaCO_3$ mg l^{-1}) and highest heavy metal concentrations (site 14, reach 2; 193 mg l^{-1} Zn and site 3, reach 2; 16.0 mg l^{-1} Cu). Field and laboratory studies (see 6.42) have shown that the organism can tolerate a combination of low pH and high heavy metal levels, although the latter showed that there were limits below which the species would not grow.

Of the physical parameters measured, speed of current was the only factor which appeared to influence growth to any great extent. Favourable current speeds have been described in the previous section, but to summarize, it seems that the combined effect of a stable substratum and mid ranged current speed (0.3 m sec^{-1}) favours good growth. The effect of scouring on E. mutabilis by faster currents (approximately 0.4 m sec^{-1}) was observed at site 4, reach 2.

Although light intensity was not measured at the reaches, as mentioned in section II, it appeared to influence the growth of the organism to some extent. Field observations showed that the cells tended to migrate away from strong light intensity. At site 16 the effect of light on the standing crop of E. mutabilis was visually obvious. Here, the density of cells was measurably greater in a stretch of the stream which was shaded by trees, compared with an area of open moorland. The results of several measurements of standing crop from the two areas over a range of current speeds is given in Fig. 7.1. The values given as less than 1000 lux were sampled from the shaded area, and those greater than 1000 lux were from the moorland section. The results demonstrate an increase in standing crop at the lower light intensity and also indicate that the maximum crop occurred at the 0.20 m sec^{-i} current speed, at both light intensities. The increase in biomass of E. mutabilis could not be attributed to any obvious changes in water chemistry or substratum, although it is possible that minor differences may have occurred.

Fig. 7.1 The standing crop of Euglena mutabilis in Dowgang burn (site 16) collected from two areas of different light intensity and at varying current speeds.



These measurements were supported to some extent by observations made during the surveys. In exposed, shallow pools, the cells tended to migrate into the upper layer of the silt substratum. However, in more exposed situations the alga also formed localised dense aggregations of continuously moving cells. Similar activities were also observed on agar plates exposed to brief periods of bright light ($\simeq$ 2000 lux).

It would seem from the data collected for Brandon Acid Stream (chapter 5) that the organism thrives best at lower pH values ($<$ pH 3.0) and also possibly where competition from other acid tolerant species is least, for example, in newly formed acid streams (eg. site 10) and where pH and other environmental conditions limit other less tolerant species. Where established mixed populations occurred, the dominance of this alga tended to decrease eg. site 3, reach 2 and 4; site 14, reach 2 and site 11.

V. The occurrence of this species in the acid habitat has been documented by many authors. Lackey (1938) found it growing well at pH 1.8 and noted that it was the most common alga at pH values below 3.0. Weaver and Nash (1968) found a species of Euglena, probably E. mutabilis, to be very common at pH values around 3.0. Bennett (1969) Steinback (1966) and Joseph (1953) all commented on the occurrence of this species. Fott (1956) reported the lowest pH at which the organism has been found, at pH 1.0, whilst Dach (1943) grew the alga at pH 1.4 in the laboratory.

7.3 Pinnularia acoricola

I. The morphological variation of this species was considerable (Fig. 7.2A) with the cell length ranging from 8 - 25 µm and the width from 3 - 6 µm. The valve, shape, area, size and general outline varied in some populations, from frustule to frustule. The small cells tended to be more rounded, whilst the larger cells (18 µm in length) tended to be elliptical. The direction and degree of curvature of the costae also altered quite markedly from cell to cell. However, the general pattern of the costae tended to be similar, with them radiating at the centre and converging at the poles. Most specimens examined had between 13 and 17 costae in 10 µm. The character which was common to all frustules was that the raphe was unilaterally bent at the central pore.

Although many of the cells from the various sites did vary considerably, some were comparatively uniform in size and shape (eg. site 4, reach 2, site 11 survey A). However, from the results it was not possible to determine which, if any, parameters were involved in producing the variability.

II. This diatom species was found to be the one most commonly occurring in streams below pH 3.0 (Table 4.4) and the only one to grow at pH values below pH 2.5. It was also the most abundant of the diatom species represented in the acid streams, and did form macroscopically obvious growth in some reaches (eg. site 4, 11, 12 and 13). It was more common and abundant in late summer than late winter.

III. There were no obvious preferences for habitat type, but the greatest density of cells was associated with acid seepages and, in some instances, the pool habitat (eg. site 4, reach 1 and 4: site 6, reach 8: site 8, reach 1). However, abundant growth was recorded in the faster current speeds (eg. site 5, reach 2: site 8, reach 4) but only where the substratum was reasonably stable.

IV. Apart from some instability of the frustules in fast current speeds, the presence and absence of this species did not appear to be controlled by the parameters measured. It grew at pH 1.5 and in the presence of large concentrations of heavy metals (see Table 4.9), although these were not the maximum values recorded.

V. This species does not appear to be distributed widely. Hustedt (1938) reported that it was a common species in Java and Cholnoky (1958) found it in South Africa. Both authors noted the acidophilic nature of the diatom and Cholnoky reported that optimum conditions for growth were at about pH 5.0 but that it grew well at pH 2.4.

More recently, Hancock (1973) reported it's presence in South Africa. Carter, (1972) described the large variation of the frustules he found in a sample taken from Brandon Pithouse Acid Stream (site 3 in this study) in 1971. He concluded that the variation was so great that the only definite factor upon which the identification could be made was the shape of raphe. There is no mention of this species in the U.S.A. literature, although several different species

Fig. 7.2 A. Pinnularia acoricola, B. Nitzschia subcapitellata, C. Nitzschia elliptica var. alexandrina, D. Eunotia exigua.

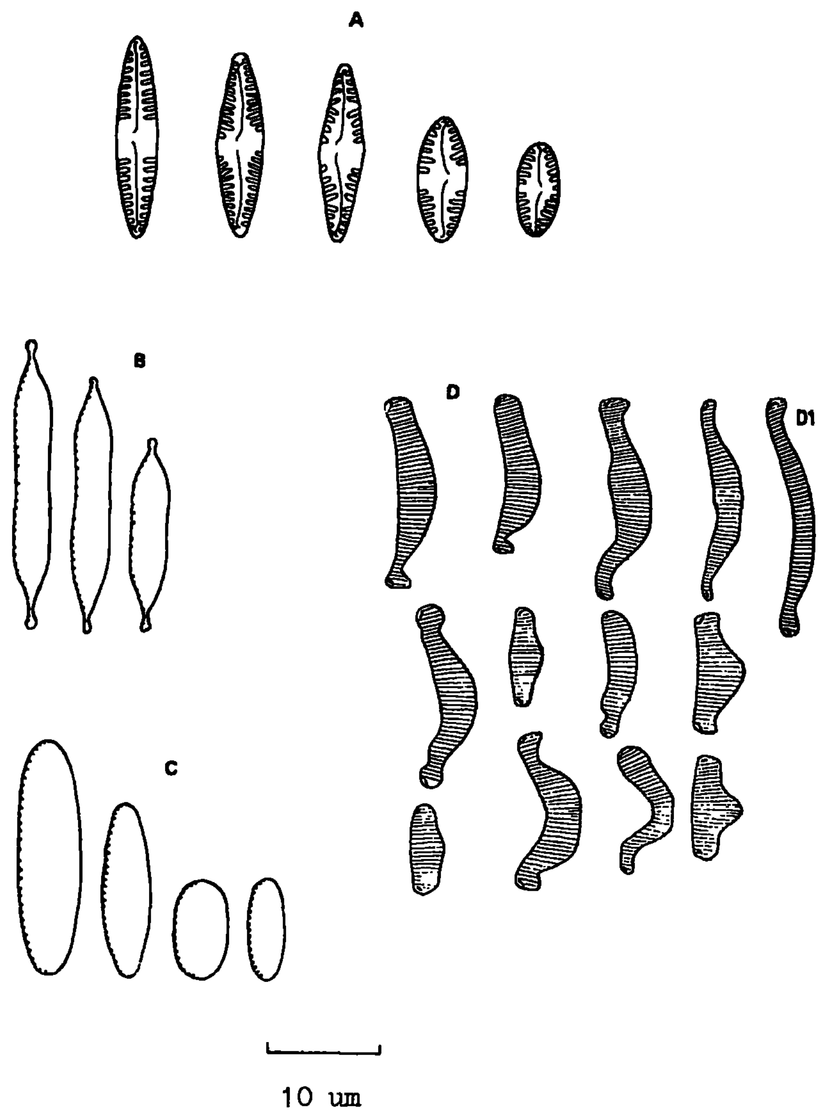


Fig. 7.3 A. Nitzschia sp. type A, B. Nitzschia sp. type B, C. Navicula sp., D. Nitzschia ovalis, E. Pinnularia microstauron

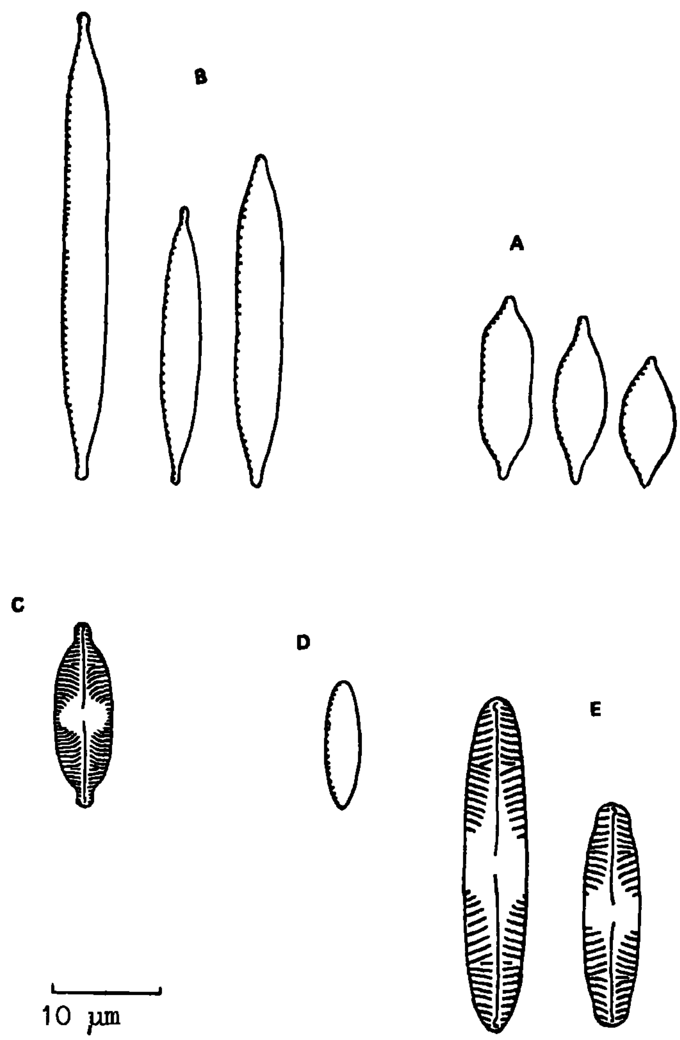
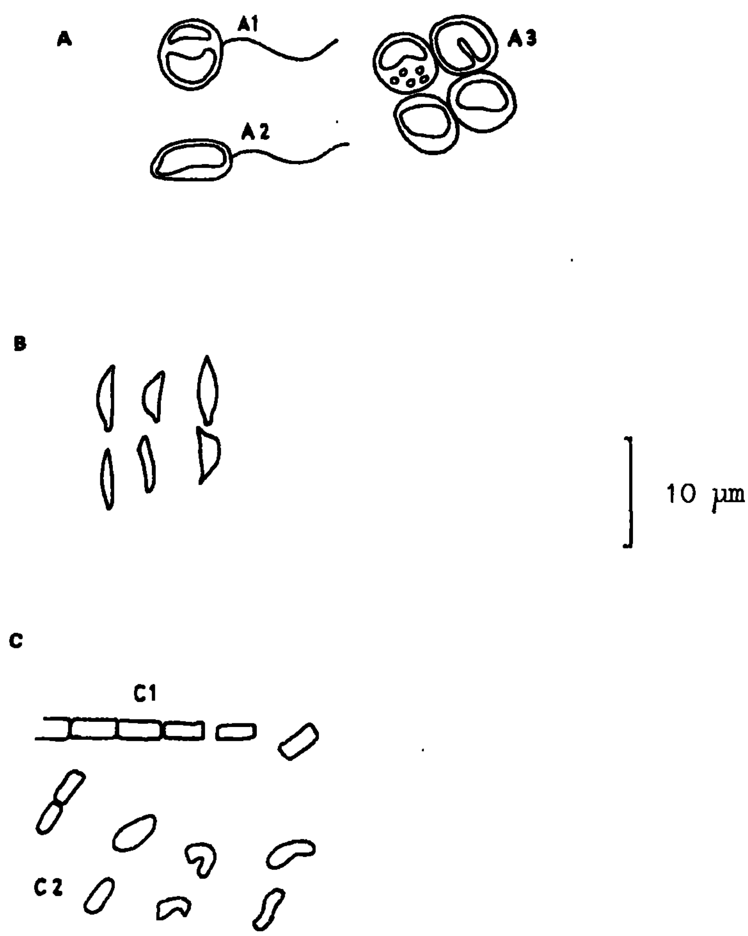


Fig.7.4 A. Gloeochrysis turfosa, B. Characium sp.
C. Stichococcus bacillaris



of *Pinnularia* have been named (eg. *P. braunii*, *P. microstauron* and *P. termitina*).

7.4 *Gloeochrysis turfosa*

I. The motile and non motile stages of *Gloeochrysis turfosa* showed some morphological variation. The motile cells were particularly variable in size and shape. These ranged from spherical, slow moving cells Fig. 7.4A1 to an oval., fast moving form Fig. 7.4A2. Both forms had a single flagellum approximately 1.5 x the cell diameter. The cell dimensions of both stages varied from 3.5 to 8.0 µm in diameter.

Although the shape of the non motile cells was comparitively constant Fig. 7.6A3, the size range was greater than that found in the motile form. The variation occurred both within one population and between populations. There was some indication, both from the field results and laboratory studies (see 6.35), that cell size decreased at the lower pH values.

Another variable feature of this species was the position of the chromatophore, which was found to be both parietal and central. Observations indicated that the parietal state was more common in the non motile stage and that the larger the chromatophore the more parietal it became. Both observations were very variable and seemed to be linked with the age of the cells, rather than environmental factors.

The aerial stage was rarely found in the surveys, although the typical brown, macrophytic growths which were sometimes observed usually occurred at the air-water interface. In culture, the motile cells tended to congregate at the surface of the medium, thus producing a thin film of cells at the air-liquid interface. On occasions when the alga was subjected to drought, there was an obvious increase in mucilage surrounding the cells and the material remained viable for several days.

II. This chrysophyte was a common member of the acid habitat and was more common and abundant in summer (see Table 4.4 and 4.7) than in winter. These observations were also confirmed by the data from Brandon Acid Stream (see 5/10). Although the species was often abundant and macroscopically obvious, it rarely dominated a reach completely, but tended instead to produce smaller localized colonies.

III. The alga was recorded in all types of habitat, but the swarmers were more abundant in pools and streams with a slow current speed (eg. sites 1, 6, 12). However, the non-motile stage was found in faster currents (eg. site 3, reach 1), possibly because the mucilage would enable the colony to withstand the scouring effects of faster water.

Both forms were often found in large numbers, in association with decaying organic material such as grass, leaves and twigs.

IV. Although the data did not indicate any relationship between the parameters measured and the occurrence of the species, there were some factors which may have influenced it's presence. Even though it occurred in abundance at pH 1.8 and grew at pH 1.5 in the laboratory (see 6.31) it was more widespread above pH 2.5. There was also some indication from preliminary laboratory studies (see 2.72) that the addition of ammonium as an alternative source of nitrogen, improved growth. Under field conditions the organism grew in a wide range of NH_4-N concentrations (0.05 - 10.3 mg l^{-1}) and whilst there was no obvious relationship between NH_4-N and the presence of the chrysophyte, the results showed that values of NH_4-N were greater in survey A when the organism was more common.

As mentioned in 6.7, a population of the alga which was isolated from Brandon Acid Stream was found to be sensitive to high temperatures (18 - 20^{o}C) and although values as high as these were not often recorded in the streams, temperature may well be a growth limiting factor.

V. Although this species was found to be a common member of the acidic environment, there are no records of it's presence at low pH. Other chrysophycae species have been recorded at below pH 3.0, Lackey (1938) and Bennett (1968) reported Chromulina ovalis as common and Lackey recorded a species of Ochromonas.

7.5 Nitzschia subcapitellata

I. The morphology of this diatom was comparatively constant when compared with some of the other diatoms. Even so, cell length varied from 12 - 30 μm and cell width from 5 - 8 μm, Fig. 7.2B. There was also some difference in the degree of polar capitation and to a lesser extent, the convexity of the frustule margins. As shown in Fig. 7.2B, one of the main characteristics of this species was the obvious separation between the middle keelpoints. This character did not vary between individuals or populations. The striae were extremely fine and were only visible under the best optical conditions.

II. The species occurred in 30% of the possible reaches in both surveys, but was rarely the most abundant organism present in any one reach. Occasionally it formed a macroscopic film over undisturbed sediment, but it's presence was not generally conspicuous. The seasonal data based on relative abundance (Fig. 5.14), showed that in Brandon Acid Stream, the species was present throughout the year, but most abundant during autumn.

III. Growth tended to be more frequent in reaches of slow current speeds (eg. site 11 and 12) but over a wide variety of substrata, including the soft flocculating type produced by continuously precipitating iron hydroxide. Although growth was never dense under these conditions, the alga seemed capable of colonizing where other non-motile diatoms did not.

IV. There was no evidence to suggest that the growth of N. subcapitellata was controlled by heavy metals, or that it's distribution was greatly affected by nutrient concentrations, however, the data strongly indicated that growth was restricted to pH values above pH 2.5. Within the pH range examined, the greatest density of cells occurred near pH 3.0 (eg. site 3, reach 4; site 6, reach 8).

V. Although a common alga in the acid streams in England, it's presence was not recorded in the U.S.A. studies. Cholnoky (1958) mentions the diatom as a reasonably common species of the brackish habitat, but does not indicate it's pH tolerance.

7.6 Nitzschia elliptica var. alexandrina

I. The morphology was very variable, both within a population and between different populations. In some cases, the variation between forms was such that it was difficult to ascertain with certainty that all the specimens were one species (see Fig. 7.2C). The previous descriptions of this diatom showed a frustule with linear sides and rounded ends, having a general hyaline appearance. The pitch of the striae was between 42 - 46 striae in 10 µm and keel points from 16 to 20 in 10 µm. Many of the specimens did fall into this general description, but the size range 8 - 20 µm and the shape, varied considerably more than normally described, as shown in the diagram. Many specimens did not have linear sides, but were distinctly rounded, this characteristic is not included in previous descriptions of the species.

It was observed that the smaller frustules were usually oval, whilst the larger ones tended to be elliptical or near linear. It is possible that this variation could be determined by the age of the clone, the larger cells being near to the auxospore stage (J. C. Carter, pers. comm.). However, the samples taken from reach 3 of Brandon Pithouse Acid Stream, over three years, were consistently dominated by smaller, oval cells. This would suggest that age was not the main factor in determining cell size and shape, but that some other factor was involved.

II. The diatom was more widespread in the late winter than late summer period (see Table 4.4). It was more abundant in winter than the summer period, but with the exception of site 3, reach 3, it was not considered to be a particularly dominant species. Observations from this reach showed that it was most abundant during the spring (see Fig. 5/30).

III. The distribution of the species was restricted to some extent by current speed and it was generally found to grow best in pools and reaches of slower current speeds (eg. site 2, 11 and site 6, reach 8.). It also had a preference for a firm substratum.

IV. The organism was restricted to waters of pH values above 2.5. However, there was no obvious relationship between morphological variation and pH, or associated chemical parameters. The levels of heavy metals in which the species was recorded were not the maximum possible (see Table 4.9) but nevertheless it was capable of growth in relatively large concentrations (site 14, reach 1; site 2, reach 1). As mentioned in section III, the reasons for the dominance by _N_. _elliptica_ var. _alexandrina_ at reach 3 of Brandon Pithouse Acid Stream were not apparent, but it is possible that it may be a light sensitive species. Reach 3 of the stream is shaded (see Table 3.3) with a substratum of predominantly small pebbles.

As the chemical composition of the water at this point was little different from the unshaded areas above and below reach 3, it was felt that the combination of a suitable substratum and reduced light may explain the abundance of the species at this point. However, at other sites where dense growth occurred, the same conditions were not always prevalent (eg. site 2, 6 and 5).

V. Prior to this study the species had been recorded in various different habitats. Hustedf (1935) recorded it in a warm spring in Africa, and Cholnoky (1958) found the variety alexandrina in a mineral lake in South Africa. J. C. Carter (1973, pers. comm.) has recorded the same variety in Britain, but not in association with mine waters, or displaying such a large variation in form.

7.7 Eunotia exigua

I. The morphology of the diatom Eunotia exigua was extremely variable, as shown in Fig. 7.2D. The size and shape of the frustules at times varied dramatically within one population (eg. site 3, 5 and 14). Cell length ranged rom 5 - 30 μm, whilst cell width remained relatively constant. An example of a normal frustle is shown in Fig. 7.2D1. The number of striae per 10 μm was relatively stable, even in the most contorted cells. The reasons for the extreme variation in form observed for this species is not known.

II. The species was widely distributed in waters of pH values above pH 2.5. The data from Brandon Pithouse Acid

Stream (Fig. 5/30) showed that it was most abundant in spring. However, it was not usually recorded as a particularly dominant species, although on occasions it was sufficiently numerous to be macroscopically obvious (eg. site 3, reach 1: sites 11 and 14).

III. The organism did not seem to have any distinct habitat preferences. The most abundant growths tended to occur in the medium and slow current speeds, especially where the substratum was reasonably stable. It was also found in association with mosses (eg. site 3, reach 1: sites 11 and 14).

IV. As the organism was not recorded below pH 2.5, it was felt that pH had some controlling influence on growth. In an attempt to ascertain whether pH, or heavy metals, were responsible for the variability of the frustules, specimens were collected from reach 1 (pH 2.6), and reach 11 (pH 3.0), of Brandon Pithouse Acid Stream, and the degree of twisting length compared. There was an obvious difference between the populations, at reach 1 the frustules were considerably more contorted and the average cell length significantly shorter, at the 99.9% level, than those from reach 11, (17.3 μm $\pm$ S.E. 0.873 compared with 27.2 μm $\pm$ S.E. 1.36). The population from reach 11 was more consistent in size and shape. These observations were found to be a permanent feature of the two populations. As the levels of heavy metals, silicate and major nutrients were similar at both sites (see Figs. 5.1 - 5/27), it would appear that the malformation of frustules could be attributed to the effect of pH.

V. Eunotia exigua appears to have a worldwide distribution in the acidic habitat. It has been reported below pH 3.0, in U.S.A., by Steinback (1966); Warner (1968); Bennett (1969) and at pH 2.6 by Besch *et al*. (1972). It was recorded in acid streams in South Africa by Cholnoky (1958 & 1960) and Hancock (1973). Besch *et al*. (1972) also noted malformed frustules in the presence of large concentrations of Cu, lead and Zn, but other authors make no mention of such features (for further discussion see 8.55).

7.8 Chlamydomonas applanata var. acidophila

I. The general description of the organism recorded here was the same as that described by Fott (1956) for Chlamydomonas applanata var. acidophila and by Negoro (1944) for the species Chlamydomonas acidophila. The two species are considered to be synomymous. The cell dimensions varied from 6.0 - 10.5 µm in length and 3.5 - 8.0 µm in width. Unlike the specimens examined by Fott, the species recorded here demonstrated the ability to produce a palmelloid stage, both in culture and in field conditions. In culture the resting state was more common in nutrient depleted media and at the lower pH values. In the latter situation it was difficult to determine whether these cells were truly in the palmelloid state, or whether they had lost their flagella, a feature which was common at the extreme pH values.

Although this species did not demonstrate any obvious morphological variations, other than those already mentioned, the shape of the cells seemed to change with their age. The freshly liberated daughter cells tended to be more narrow and pointed at the apex, whilst the older cells were large, ovoid and with a more rounded apex. These variations in shape with age have already been recorded for this genus by Lund (1947).

II. The alga was both more common and more abundant in the summer than winter (see Table 4.4) and on occasions was found to produce a macroscopically obvious bloom (eg. site 9, reach 5; site 13, reach 1).

III. It was generally independent of the substratum because of it's motility. The greatest densities were observed in the pool habitat and reaches of very slow current speeds (eg. sites 9, 13 and 14). Where it did occur in the faster current speeds, it was usually associated with mosses or filamentous algae (eg. site 3, reach 1: site 4, reach 3).

IV. There was no evidence to suggest that it's presence was contolled by any single physical or chemical factor. In culture, growth was increased by the presence of ammonium salts, but the field data did not indicate that NH_4-N (see 2.72) values were correlated with its presence or abundance. Hopkins and Wann 1926 also found that a species of <u>Chlorella</u> utilized NH_4 in preference to NO_3.

It was very tolerant to low pH, both in the field (eg. site 4, reach 7) and laboratory (see Fig. 6.3). It was also resistant to large concentrations of heavy metals (see Table 4.9).

As mentioned in 6.31, the organism demonstrated the ability to reduce the pH of the medium down to values around pH 2.0. This phenomenon has also been found to occur with the thermophilic alga Cyanidium caldarium, but no explanation of this ability is known. Hawley (1971) suggested that oxidation of ferrous sulphate by bacteria, may result in the production of sulphuric acid and thus a lowering of the pH, however, there was no indication that Chlamydomonas applanata was utilizing the iron in solution.

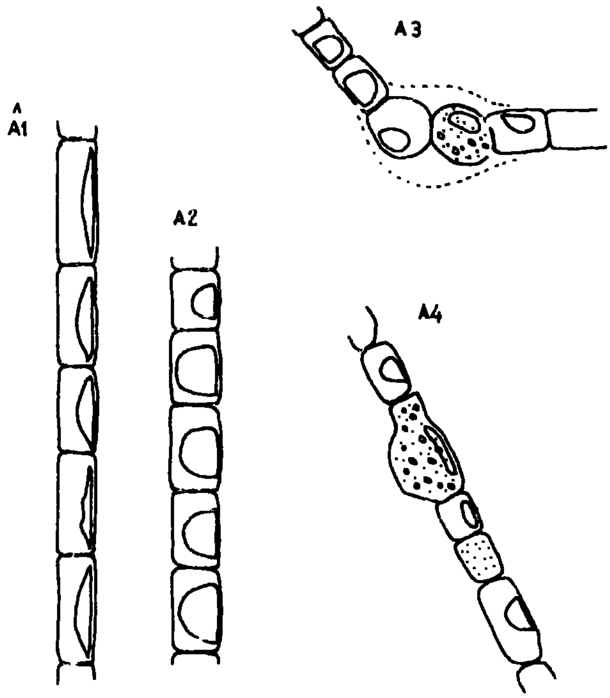
As there was such an obvious seasonal growth cycle for this species, as confirmed by the Brandon data (see Fig. 5/30), it would suggest that temperature may also be important in controlling it's growth (Fig. 6/11).

V. Many of the authors investigating the acid habitat reported the presence of species of Chlamydomonas, for example Steinback (1966), Lackey (1938) and Bennett (1969). Fott (1956) found Chlamydomonas applanata var. acidophila growing well between pH 1 and 2 and Negoro (1944) recorded the synonymous species Chlamydomonas acidophila, at pH 1.7 in a Japanese Lake.

7.9 Hormidium rivulare

Although the generic name Hormidium was changed by Fott (1960) to Chlorohormidium because a genus of orchid has priority for the former name, until the current critical studies of the genus have been completed (Pickett-Heaps, 1972), the traditional generic and specific names given by Heering (1914) have been retained in order to

Fig. 7.5 Hormidium rivulare A1 and A2 demonstrate variation in chloroplasts, A3 geniculation, A4 distorted filament with heavy granulation.



ovoid confusion. The variation in the diagnostic characteristics of *Hormidium rivulare*, under different environmental conditions, was at times confusing and often only with the assistance of culturing in a standard medium could the identification be completed.

As shown in Fig. 7.5 the cell breadth and length varied considerably both between populations and within the length of one filament. Cell breadth ranged from 5 - 10 µm with a mean of 7 µm and cell length varied between 1 and 3 times the breadth. The longer cells also tended to be thinner (Fig. 7.5A1), although this was not always found to be the case. The longer cells seemed to be indicative of a healthy, but slower growth rate. This condition was often found in slow flowing waters and seepages where the filaments were longer, probably due to minimal mechanical breaking up of the filaments.

The thickness of the cell wall was also variable and altered with the environmental conditions. At the lower pH values and higher heavy metal concentrations, the cell walls were often considerably thicker. This observation was also made on laboratory cultures grown at low pH and in large concentrations of Zn and Cu (see 6.35 and 6.4).

Two characteristics of the species are the presence of constrictions between cells, and the presence of mucilage. Both of these varied considerably with environmental change. The constrictions were usually well defined, but in filaments from a population growing in presumed optimum conditions, they were often difficult to detect (see Fig. 7.51A). Although it is difficult to assess the amount of mucilage present around a filament, observations indicated that mucilage production increased when the organism was growing in the lower pH conditions (pH 2.75 - 3.0), but that at pH the limit for growth, estimated to be pH 2.5 (see 6.35), the amount of mucilage was markedly reduced.

The single parietal chloroplast of each cell was usually clearly defined and occupied between 0.25 and 0.45 of the cell volume. From a transverse view of the cells, the shape of the chloroplast varied from semi-elliptical (Fig. 7.5A1) to semi-circular (Fig. 7.5A2). The ellipitical form was generally associated with the longer cells, whilst the rounded chloroplasts were predominantly found in the shorter cells and therefore could be determined by the age and state of the cells. It was not unusual to find examples of both types in one filament, although usually one was the dominant type for a given filament.

In field and cultured populations when growth was rapid, filaments with short cell length and rounded chloroplasts tended to be dominant, but in populations in which the growth rate was slower, the occurrence of filaments with longer cells and ellipitical chloroplasts increased. Under conditions of low pH (pH 2.5) and large concentrations of heavy metals, both field and laboratory material included filaments in which the cells were often distorted and enlarged with thickened cell walls, and invariably the whole filament was twisted (Fig. 7.5A4). The chloroplast margins became indistinct and the volume of cell occupied by the chloroplast was reduced to less than 0.25 total cell volume. The amount of granulation in these cells also increased, indicating that normal metabolism was being inhibited. The decreased growth which occurred in sub-optimal pH and heavy metal growth conditions, was evident by an increase in the frequency of longer, narrower cells with semi elliptic chloroplasts.

The occurrence of geniculations (Fig. 7.43) is considered to be an important diagnostic feature of Hormidium rivulare Heering (1914) and although they occurred in every population, their frequency was extremely variable. Again, conditions which inhibited growth seemed to cause an increase in geniculations and an increase in mucilage around the knee joints, as shown in 7.5A3. They were also more common in healthy populations dominated by long filaments and hence may be indicative of older populations. However, in rapidly growing populations their presence was found at times to be extremely rare.

The formation of akinetes was common for this species, and the occurrence of zoospores was never observed. Akinete formation was most common in older populations and in conditions where growth was inhibited. Fragmentation may also be considered important as a method of dispersal for this species. Usually the filaments separate at the geniculation where it is present, but fragmentation also occurred at any point along a filament if mechanically broken .

II. The occurrence of this alga was widespread above pH 2.5. From the survey and Brandon Pithouse Acid Stream data, it was found to be most common and abundant in summer and autumn. Where the species occurred, it tended to be the dominant or co-dominant alga, often completely covering the stream bed (eg. site 3, reach 4: site 8, reach 3: and site 12). It's optimum growth pH under laboratory conditions was found to be pH 3.5 - 4.0 and under field conditions its growth was obviously inhibited by values of less than pH 2.7.

III. The distribution and abundance of the alga was not affected to any great extent by current velocity, but its occurrence in stagnant water was rare. The type of substratum did not appear to be restrictive, as long as there was a stable substratum which would prevent the filaments being washed out in fast current speeds.

IV. As mentioned in 6.34, the lowest pH at which this species was found to grow either in the field or artificial medium was pH 2.5. It was therefore assumed that this pH value was near it's limit of growth and that pH may be one of the major factors controlling it's presence and abundance in the acidic environment.

The alga was also capable of tolerating quite high concentrations of heavy metals. The survey data showed that whilst it grew well in high levels of Zn (maximum tolerated 67.4 mg l^{-1}), it did not grow in the reaches where higher levels occurred (see Tables 4.9). The absence of the species at the higher levels may be due to the habitat being unsuitable, rather than the level of Zn restricting growth. For example, at site 14, the alga was abundant in reach 1 at 67.4 mg l^{-1} Zn, but absent in the pool habitat of reach 2 at 193 mg l^{-1} Zn. Laboratory studies on one population of _Hormidium rivulare_ showed that the growth of this population was restricted to only 30 mg l^{-1} Zn, but that levels of less than those above were toxic at high pH values. It would therefore appear that both pH and heavy metal concentration have some influence on the growth of this species and that resistance to heavy metals varies from one population to another.

V. Apart from this study there appears to be no other record of this species at low pH. However, several authors have reported other species which possibly include _Hormidium rivulare._ For example, Shellgren _et al_. (1967) reported _Hormidium_ sp. as the most common species between pH 3.0 and 3.7. Weaver & Nash (1968) recorded a similar species _Hormidium subtile_ growing at pH 3.0 and 3.5.

7/10 _Zygogonium ericetorum_

I. Of the species recorded below pH 3.0, _Zygogonium ericetorum_ was one of those which demonstrated the least morphological variation. The most variable diagnostic characteristic was the cell wall thickness, this increased when growth conditions were unfavourable, for instance at growth limiting pH. Under inhibitory pH conditions, estimated to be around pH 2.5 (see Tables 4.7 and 4.9) the cell wall thickening was often accompanied by a decrease in chloroplast size. Around pH 2.5 the above morphological changes were followed by further distortion of the cells and eventual death.

Many of the populations recorded were dominated by cells containing a purple-brown non-chloroplast pigment, which is a characteristic of the species. The occurrence of the pigment did not seem to be linked with any chemical factor (eg. Fe). However, it was observed that in cells that were shaded by a mass of other filaments they did not contain the pigment as frequently as those that were in full sunlight. This observation was partly supported by a decrease in the depth of colour of the pigment when the filaments were kept in the dark for 24 hours.

II. The alga did not appear to have any obvious seasonal cycle and was common and abundant in both surveys. Where it occurred, it was invariably the dominant species, often in association with _Hormidium rivulare_ and forming a thick mat of filaments.

III. It was mainly found in the slow flowing and stagnant sites (eg. site 1: site 6, reaches, 1,2), but once established, it was capable of withstanding faster current speeds. It was often abundant in the acid seepages (eg. sites 1 and 8).

IV. As already mentioned in section I, the lowest pH at which the species was found growing was pH 2.5. However, on occasions, live filaments were recorded at slightly lower pH values, but observations suggested that the alga could not grow permanently under these conditions, as many of the cells were dead and those which were alive were distorted and in many cases the chloroplast was yellowed. The alga seemed capable of tolerating large concentrations of heavy metals, in particular Zn , as well as high acidity (eg. site 14, reach 2: site 6, reach 1, 2).

V. the only reference to this species was made by Weaver & Nash (1968) at pH 3.0 to 3.5.

7.11 *Characium* sp.

I. The morphology of this alga was very variable (see Fig. 7.4B) and it was not possible to designate a binomial. Attempts to culture the alga in order to produce a more uniform population, were unsuccessful.

The cell lengths ranged from 2-6 µm and width from 2-3 µm. The chloroplast was parietal, and often filled three quarters of the total cell volume. The cell shape varied considerably, from ovoid, to almost semicircular. There was no obvious basal attachment on most of the cells, although occasionally, this was observed (eg. site 1, reach 3).

II. The species was more common in summer than winter, but was rarely abundant.

III. The alga was most widespread in slow flowing waters and in association with moss and filamentous algal species, but it was also found to colonise bedrock in quite rapid current speeds (eg. site 12).

IV. Although there was some indication that at the more extreme pH values the morphological variation increased, the alga did not seem to be influenced by any other parameter measured. It was very resistant to low pH (lowest pH at which growth was recorded was pH 1.5) and high acidity, and also to large concentrations of heavy metals (eg. site 4, reach 5: site 12, reaches 2, 3).

V. ---

7/12 Lepocinclis ovum

I. This alga was not found to vary much in size or morphology, when compared with other species recorded here. At pH 2.5 the occurrence of 'monster' cells (see 7.2) was quite common.

II. It was only recorded in the winter survey at six reaches in three sites. It was reasonably abundant in some of these reaches (eg. site 6, reaches 7, 9: site 8, reach 1). The seasonal cycle, as indicated by the surveys, was not found in Brandon Pithouse Acid Stream (site 3, reach 10) at pH 3.2. At this reach the alga was recorded from July to late October, but not during the winter and spring seasons.

III. It was most abundant in slow flowing waters and pools (eg. site , reach 1: site 8, reach 1) and did not appear to be restricted by the type of substratum.

IV. Although there were only a few recordings of this species, the data suggest that pH 2.5 may be it's growth limiting pH. In Brandon Pithouse Acid Stream it was never found growing below pH 3.0, although the opportunity to to colonise lower pH values was available. As mentioned in section III, current speed also appeared to influence it's presence in a reach.

V. Lepocinclis ovum was reported by Lackey (1938) as growing at pH 4.5, but was not recorded at lower values even though they were available for inoculation by the species.

7/13 Chlamydomonas sp.

I. This species was recorded separately from Chlamydomonas applanata var. acidophila mainly because of the size difference. The cell demensions were: length 2-3.5 μm and breadth 1.5 - 3.0 μm. The two flagella were 2-3 times the cell length and the cells were ovoid to round, with a point at the apex, the chlorplast was parietal with a stigma near the apex and a single pyrenoid, which at times was not very obvious. It is possible that this species was a variety of C. applanata, however, it was felt that it's pH characteristics and general appearance were sufficiently different to justify its inclusion as a separate species.

II. It was recorded at five reaches in two sites and was abundant in two of those reaches (site 6, reaches 7 and 9). Like the other chlamydomonad, the few records collected suggested that it was more common in summer than winter.

III. As with most of the flagellated species it was only found in the slower flowing streams and the pool habitat.

V. It was recorded at and above pH 2.7, but there are insufficient records to make any judgements on it's tolerance to pH or other chemical factors recorded.

V. ---

7/14 Stichococcus bacillaris

I. At pH values above 2.0 Stichococcus bacillaris had uniform morphology (Fig. 7.4C1). Below pH 2.0, both in culture (see 6.35) and in field samples (eg. site 4, reach 7) cells were mis-shapen, varying from the normal rod, to ovoid, 's' and 'u' shaped cells (see Fig. 7.4C2). The chloroplast did not appear to be affected in any way by the low pH, although at pH 1.5 in culture, the chloroplast was reduced and yellow. Although the growth rate was reduced at pH 1.5 (see 6.35) these distorted cells seemed capable of division and as already mentioned in 6.31 at pH 1.75 growth was not reduced significantly, even though the population was dominated by distorted cells.

II. As previously described in 4.41, it was not a common species in the acid habitat, occurring at only four sites and only one of these on both surveys (site 3, reach 1). It was never an abundant species (see Table 4.8) although at site 3, occasional macroscopic growths were observed.

III. It was mainly found in pools and slow flowing streams, but single cells were found in association with other plants in faster current speeds (eg. site 3, reach 1).

IV. It was very tolerant of pH both in the field (pH 1.8) and in the laboratory (pH 1.5) and was also recorded at high acidity values (eg. site 4, reach 7).

V. No records of this species in the acid habitat were found in the literature, although reference was made to a morphologically similar species, Stichococcus subtilis in a report on acid pollution by Patrick (1974).

7/15 Nitzschia sp. type A

I. This species varied considerably in morphology as shown in Fig. 7.3A, with the length of the frustules ranging from 10-20 μm and the width from 6-7 μm, the keel points were between 13 - 16 in 10 μm and the striae were indistinct under a light microscope. The central keel points were slightly separated and the frustules capitate. The latter character was very variable.

It was not possible to find a description which fitted this diatom completely. The size, the number of keel points and the striae resemble those of Nitzschia pseudofonticola, but the separated central keel points are not characteristic of this species.

Some of the frustules were similar to the extreme forms of Nitzschia subcapitellata, however, other characteristics such as keel points and degree of capitation do not fit the description of N. subcapitellata. It is possible that this may be an undescribed species, or it may just be a variety of an existing species, possibly of the N. fonticola group (J. C. Carter, pers. comm. 1973).

II. Numerous specimens of this species were recorded from two different sites in survey A (sites 1 and 11) but were not recorded at any other reach or in the same reaches in survey B.

III. Both sites were characterized by slow flowing and stagnant water, with a silty substratum.

IV. Neither of the sites at which the alga was recorded were particulary rich in heavy metals and the lowest pH value was pH 2.5.

V. ---

7/16 Cryptomonas sp.

I. Because the occurrence and abundance of this species were low, it was not possible to gain much information on the morphology of the organism. The nearest description was to that of *Cryptomonas erosa*, but because it was not possible to culture the alga and only the occasional cells were found at any one time, accurate identification was not possible.

II. The alga was only recorded at two sites (sites 3 and 11), one in each of the surveys. At site 3, Brandon Pithouse Acid Stream, it occurred throughout the year but never in large numbers.

III. It grew in flowing and stagnant waters, in the former it tended to be associated with mosses.

IV. ---

V. Both *Cryptomonas erosa* and *C. ovata* have been recorded below pH 3.0 in the U.S.A. surveys of Lackey (1938) and Bennett (1969).

7/17 Nitzschia ovalis

I. Only a few live specimens of this species were found at two sites and there was no indication of morphological variation. It is possible that this species may be a variant of Nitzschia elliptica, as Nitzschia ovalis is usually found in brackish and marine environments. However, the oval shape and very fine striations normally ascribed to N. ovalis were present (see Fig. 7.3D) and the number of keel points were also characteristic. Nevertheless the form already described for N. elliptica (see 7.6) was sufficiently diverse as to include the characteristics of N. ovalis. N. elliptica was not recorded in the same reach, at the same time, (site 6, reach 8), but was found in the subsequent survey, thus placing further doubt on the identification of Nitzschia ovalis.

II. The diatom was only recorded at two sites (site 6, reach 8: site 11, reach 1) and was not found in large numbers. No records were made in the second survey, thus suggesting that it was more common in the late summer than winter period.

IV. Both records of the diatom were made in the pool habitat comprising a clay and silt substrata.

IV. The lowest pH at which it occurred was pH 2.5 and there did not appear to be any more variation in morphology than at pH 2.8. The heavy metal concentrations in these waters were low for acid streams, therefore it's resistance to these metals in unknown.

V. It does not appear to have been recorded by any other author at low pH.

7/18 Pinnularia microstauron

I. The morphology of the few frustules found of this species was in keeping with the previous descriptions given in the literature, although there was quite a large variation in size and to a lesser extent outline (see Fig. 7.2E).

II. It occurred at site 11, reach 2, in both surveys. Only a few specimens were collected on each occasion, but all were alive at the time of examination.

III. The site at which they were found was a large pool with a silt clay substratum.

IV. ---

V. Although this species was not reported in the literature as growing below pH 3.0, it was recorded at higher acid pH values by Hancock (1973) and Besch et al. (1972).

7/19 Nitzschia palea

I. The specimens collected were characteristic of the species and there appeared to be no morphological variation within the population sampled.

II. The diatom was only recorded at site 3, reach 4, on both surveys. It was more abundant during the late summer survey.

III. The alga was growing in a slow flowing reach of the stream in association with Drepanocladus fluitans. The substratum consisted of clay and silt, with some shale occurring.

IV. The lowest pH at which it occurred was pH 2.7 and the heavy metal concentrations in the water were low for this habitat.

V. Reports of the occurrence of this diatom at pH 3.0 and above are given by Bennett (1969), Patrick (1974) and Hancock (1973).

7/20 <u>Navicula</u> sp.

I. The morphology of all the specimens collected was considerably more constant than most of the diatoms recorded. There does not appear to be a description which totally accommodates this species and it may eventually prove to justify a new binomial. The length varied from 14.5 - 16 µm and the width 4.5 - 6 µm. There were 26 - 28 striae in 10 µm, with a circular, clear area in the central area of the frustule (see Fig. 7.3C). The striae at the ends of the frustules were closely pitched and linear, whilst towards the centre they became increasingly shorter and bent in towards the centre.

The closest affinity of this species to any previously described was to <u>Navicula</u> <u>descussis</u> which has a similar size range and striae formation. However, the central areas of the frustules of both species were sufficiently different so as to separate them.

II. A few frustules of this diatom were found in both surveys at site 11, reach 1. Some dead frustules were also recorded at site 9, reach 3.

III. The site at which the live cells were recorded was a pool habitat.

IV. It is possible that the growth of this species may be restricted by low pH, as it grew at pH 2.5 and 2.8, but all the frustules were dead at pH 2.1, at site 9.

V. Although no description of several *Navicula* spp. are given, Lackey (1938) and Steinback (1966) recorded species of *Navicula* below pH 3.0.

7/21 *Nitzschia* sp. type B

I. There was not a description which completely fitted this *Nitzschia* sp. and it may possibly be a new species (see Fig. 7.3B).

The length varied from 20 - 60 µm and the width from 4 - 6 µm; there were between 13 - 15 keelpoints and 42 - 45 striae in 10 µm. The central keelpoints were obviously separated, thus forming a link with type A. The sides of the frustules were parallel, but some were either slightly convex or concave. The degree of capitation varied considerably, with the longer frustules tending to be more capitate than the smaller form.

II. This species was only recorded at site 11, in survey A, but there were sufficient live specimens collected to include it as a living member of the diatom population.

III. As mentioned above, the diatom was growing at site 11, reach 1 which was a large pool (see Table 3.1) with a silt and clay substratum. Because of it's size it was assumed that the pool was likely to be permanent and therefore provided a stable habitat.

IV. ---

V. ---

7/23 Navicula nivalis

I. Only a few specimens of this diatom were obtained and these did not exhibit any gross morphological variation.

II. The diatom was recorded only at site 5, reach 3. Although there were quite a number of specimens, no cells were recorded during the second survey.

III The alga was growing in a medium current speed (0.27 m sec^{-1}), on a substratum of predominantly shale and clay.

IV. ---

V. No previous records of the presence of this species in the acid environment were found.

7/23 Ulothrix zonata

I. No morphological anomalies were evident.

II. The species only occurred at reach 1, site 12, in the late winter survey. The filaments were growing with a population of Hormidium rivulare.

III. The filaments were growing over a firm clay substratum, in slow flowing water.

IV. There was no evidence that the growth was affected by pH 3.0 and moderately large concentrations of heavy metals (see Table 4.9).

V. It was recorded by Lackey (1938) in water at pH 2.5 and as a rare member of acid creeks below pH 3.0, by Bennett (1969).

7/24 Microthamnion strictissimum

I. The specimen collected did not show any marked morphological variation, although the chloroplasts in many cells were yellow.

II. It was only recorded at site 12, reach 1 during survey A. Only a few filaments were found growing in association with <u>Hormidium rivulare</u>. It was also found in Brandon Pithouse Acid Stream at pH values of 3.1 and above, but never in the more acidic reaches.

III. In Brandon Pithouse Acid Stream it was recorded in slow and fast current speeds, and seemed capable of growth in the presence of flocculent iron oxide precipates.

IV. pH values of less than 3.0 appear to be detrimental to the growth of this alga. The Brandon data suggest that a value around pH 3.0 is the growth limit but that the alga is capable of withstandinga period of time at slightly lower pH values.

V. There are several references to the growth of this alga in the acidic environment. Bennett (1969) recorded it as rare below pH 3.0, but frequent at higher pH values. Weaver and Nash (1968), Dinsmore (1968) and Warner (1968) all recorded it present at pH values between 3.0 and 4.0.

7/25 <u>Drepanocladus fluitans (adult and protonema)</u>

I. No morphological variations were observed in either of the life stages recorded. Identification of the protonema was achieved by transplanting some protonema from the stream on to moist agar plates, containing media. After several weeks, sufficient leaves had developed to enable identification of the moss.

II. The adult moss was recorded at site 3 and site 11 in both surveys, whilst the protonema was only recorded at site 3. Both forms were abundant and tended to dominate the photosynthetic population where they occurred. At several reaches down Brandon Pithouse Acid Stream (site 3) both stages grew in close proximity, but never actually together. The protonema invariably dominated the mid-stream area, whilst the adult tended to grow from the edge of the stream outward towards midstream.

III. At both sites where the moss occurred the substratum was firm and stable, consisting of clay and compact silt. It grew in both stagnant and flowing waters, although the protonema was more abundant in the faster flowing reaches, (eg. site 3, reach 2).

IV. As mentioned in 4.41 and 8.32 the occurrence of the protonemal stage in such large quantities seems to be common in this type of habitat; however, the reasons for such a large amount of protonemal growth are not known. Rather than discuss the factors influencing presence of this species in a reach, it was decided that observations and possible mechanisms for the inhibition of the transition from the protonema to the adult would be considered here. Many of these comments also apply to <u>Dicranella</u> sp.

It is likely that pH plays some role in the inhibition of the transition from protonema to adult. The pH growth range for the adult, as seen in Brandon Pithouse Acid Stream, was from pH 2.6 to 6.0, whilst the protonema only grew from pH 2.6 to 3.2 in the field. This implies that the protonema was restricted to the more acidic reaches. However, at reaches 1 to 6 in Brandon Pithouse Acid Stream A, both forms grew at pH 2.6. At reach 2 of this stream, where the current speeds were constantly fast (0.55 ms^{-1}), the protonema virtually covered the stream bed and the adult was completely absent. As mentioned in 3.23, prior to this study, this area had been up to 0.5 m deep in the adult moss (B. A. Whitton pers. comm.), but this material was removed after a flood. Following the excavation of the stream bed, the protonema quickly covered the area and has remained dominant ever since. These observations would suggest that the scouring action of the fast current speed was responsible for preventing the development of the adult moss. However, this theory was not substantiated by reaches 5 and 6, where both forms were removed by flooding, but subsequently have recolonized the area. This suggests that current speed is not directly responsible for the inhibition of the formation of the adult form, but that the protonema is more resistant to scouring, being closer to the substratum.

One possible theory is that for the protonema to develop into the adult it must be out of the acidic environment, but at the edge of the water where it remains sufficiently moist so as to enable growth to proceed. Once the transition has taken place and the adult form is established, it is then capable of tolerating low pH as seen at reach 1. This theory is partly supported by the occurrence of newly formed adult moss growing at the edge of the streams, but rarely in the water, whereas the older material grows successfully in the low pH water.

However, it was felt that it was not solely the effect of low pH, or the heavy metals associated with the acid habitat, which prevented the transition, because the protonema grew successfully at pH 6.0 in liquid culture, without developing a single leaf. It may be that the protonema requires a period when it is emergent before the leafy stage develops, as occurred on the moist agar cultures.

IV. No records of this species were found in the literature at such low pH values, although it is considered to be an acid loving species (Watson, 1968).

7/26 *Dicranella* sp. (adult and protonema)

I. The species present at site 3, reach 4, has been identified as *Dicranella palustris*. Unfortunately, when the surveys of the acid streams were conducted, the moss was identified wrongly as *Campylopus flexuosus*. Whilst it is almost certain that all the populations recorded as *Campylopus* were *Dicranella*, it was not possible to designate the binomial with the same degree of certainty. Therefore, whilst it is likely that all the populations were *Dicranella palustris* they are reported here just as *Dicranella* sp. The protonema was again identified by culturing on moist agar plates until the adult form was produced.

II. The protonema was more common and abundant than the adult form (see Table 4.4). However, where either stage appeared they were usually the dominant species. (eg. site 1, reach 2: site 11, reaches 1, 2: site 14, reach 2). The adult was more prevalent near the edge of the stream and was rarely found in the deeper water, whereas the protonema grew extensively on the bottom of pools (eg. sites 11 and 14) often forming a thick mat.

III. Both forms of the organism were capable of growing in a range of current speeds. In the faster current speeds the protonema formed tight tufts which often represented several seasons growth. The tufts were capable of withstanding the scouring effects of floods and also dessication during summer low flows (eg. site 16). Under these conditions the organism favoured a firm substratum such as clay and boulders (eg. site 5, reach 2), whereas in the pools the substrata did not appear to be as important.

IV. The results indicate that pH influences the growth of this organism. The lowest pH at which either form was recorded was pH 2.6. In culture and in the field, where the filaments had been exposed to lower pH values, much of the moss was dead.

As mentioned in section IV of Drepanocladus fluitans, the reason for the protonemal dominance is unknown and many of the comments made there also apply to Dicranella sp.

V. No records of the occurrence of this species in the acidic habitat were found in the literature. Negoro (1944) reported the bed of an acid lake (pH 3.0) in Japan being covered by the moss <u>Rhynchostegium spiralifolium</u>, as was recorded at several sites in this study for <u>Dicranella</u> sp.

7/27 <u>Typha latifolia</u>

I. ---

II. Large numbers of plants were recorded at site 11, reach 1 on both surveys. Many of the plants were in flower during survey A.

III. Site 11 consists of a large silt and clay based pool, which is likely to provide a stable habitat throughout the year.

IV. Although the population at site 11 was healthy at pH 2.5, another population at site 6, reaches 1 and 2 was completely dead at pH 2.7. The reasons for the death of the second population are not clear, but it is possible that the higher heavy metal, Fe, Al and acidity values (see Table 4.1) may have caused the death of the population. It is also possible that the population at site 11 may have had a longer time in which to develop a resistance to the acidic environemnt, as it seems that the site has been in existence for many years, whereas the acid pond at site 6 is a more recent formed site. It is also not known whether [illegible] site 6 fluctuates to even lower values.

V. Harrison (1958) reported the occurrence of this species in South Africa and noted that it was abundant in acid waters between pH 3.7 and 5.0. Bell (1956) also recorded growths around the stripmine lakes at pH 3.0.

7/28 Juncus effusus

I. ---

II. This species was recorded growing in the acid water at site 3, reach 4. It was also found growing at higher pH values at several reaches down Brandon Pithouse Acid Stream.

III. ---

IV. There was no evidence that a pH value of 2.7 had any detrimental effect on the growth of the plant. Where the current speed was slow, the plant grew into the middle of the stream, but in faster reaches it was confined to the edge.

V. No other records of Juncus effusus were found in the literature below pH 3.0. However, Harrison (1958) did record several other species of Juncus at pH values below 4.0.

8. DISCUSSION

8.1 Geography and topography of sites below pH 3.0

The surveys carried out in the study of acidic waters at and below pH 3.0 showed that they are geographically widespread throughout England (see Fig. 3.1). Whilst it is likely that other waters do exist below pH 3.0, it was considered that the 13 sites recorded constituted the major permanent sources. As previously mentioned in 3.1, all but one of the permanent sites were associated with past and present coal mining, a feature which was also characteristic for the majority of the low pH streams so far studied in the U.S.A. and Canada (see 1.21).

From the topographical data (Table 3.1) it was shown that most of the streams were relatively small and less numerous compared with those recorded in the U.S.A. (Kinney, 1964). It is likely that the larger pools mentioned in 3.1 provided a more permanent habitat during long periods of low rainfall, than did the smaller pools and those streams which originated from seepages (eg. sites 10 and 13). It is likely that those streams which originated from springs would also be less susceptible to periods of low rainfall and therefore would provide a more stable habitat (eg. site 3 and 5). Braley (1954) showed that acid sources from spoil heaps were more susceptible to seasonal fluctation than those originating from mine shafts. Many of the streams which had pH values around pH 3.0, also had a layer of ferric hydroxide precipitate overlaying the 'natural' substratum (see 3.22). However, at some sites (eg. sites 6, 10 and 13) where the water was deep red, due to large concentrations of Fe in solution, some of that Fe had precipitated out at pH values below pH 3.0. This was presumably due to oxidation of the ferric sulphate, which occurs at a slow rate, at pH values between 2.0 and 3.0 (Hawley, 1971).

8.2 Physical and chemical aspects

8.21 Chemical characteristics

Apart from their low pH, chemical analysis of these mine waters showed that they were characterized by high levels of acidity, Mn, Al, heavy metals, SO_4-S and Si. Of the heavy metals analysed, Fe, Zn, Co and Ni were present in large concentrations. In contrast, the levels of Pb were always low compared with the other heavy metals. Hawley (1971) suggested that "the list of possible ions present in acid mine drainage waters was endless". It was assumed that the majority of the iron present in the water was in the form of ferrous, although it is probable that some ferric salts would also be present (Lundgren *et al*. 1971) particularly near pH 3.0.

Although there were examples of moderately hard waters, (eg. site 1) the acid waters were generally considered to be soft. The concentrations of phosphate and inorganic nitrogen were often sufficiently large as to be indicative of a moderately eutrophic environment, although examples did occur where phosphate levels were low (eg. sites 3, 8 and 11). Other characteristics which are not confined to the acid environment, but are nevertheless usually associated with it, were moderately low oxygen content and high optical density values.

Apart from the acidity determinations, no specific attempt was made to determine the buffering characteristics of these waters. It is likely that below pH 3.0 the normal buffering systems provided by hydroxide, carbonate, bicarbonate and phosphate ions would not be operative. However, the fact that at the same pH values, there was a range of acidity values, suggests that there was some buffering capacity present. It is likely that besides the hydrogen ions, acid salts, for example, ferrous, ferric aluminium; sulphates and silicates, would provide some buffering.

8.22 Physical characteristics

Characteristics similar to those discussed above for the chemical aspects of acidic waters, were not observed for the physical parameters measured. The occurrence of above average winter temperatures at a few sites (eg. site 1 and site 12, reach 2) due to tips burning underground, is a characteristic of some mine waters, but not necessarily the low pH habitat. Likewise, scouring due to run-off from the tips, affects all streams which drain spoil heaps.

8.23 Brandon Pithouse Acid Stream

Additional observations which may be of general significance, were made from the study of the pH gradient down Brandon Pithouse Acid Stream, which could not have been shown from the survey of waters below pH 3.0. In general, there was a decrease in the concentration of many of the parameters measured, with an increase in pH (see 5.31). This decrease was most obvious with those ions which are likely to be associated with the production of acid from the oxidation of pyrites, ie. Fe, Al, Mn, SO_4-S and Si (see 1.23). Unlike many of the sites sampled during the surveys, the chemistry of Brandon Pithouse Acid Stream was relatively stable, except when the flow was interrupted by man (see 3.5). As mentioned in 1.23, 3.22 and chapter 5 one of the most obvious effects of increasing the pH above pH 3.0, is the precipitation of ferric hydroxide from solution, onto the bed of the stream. The effect was most noticeable at reaches 8 and 13 of Brandon Pithouse Acid Stream, where the concentration of iron in solution was reduced by over 75%. Other elements were also precipitated out of solution, but to a much smaller extent (eg. Al and SO_4-S). This was because pH values between 3.0 and 4.0 were sufficiently low as to keep most ions in solution even in the relatively large concentrations at which they occurred.

8.24 Seasonal variations

Comparison of the levels of ions in the late summer and winter (see Table 4.2) showed that there were more higher values in winter than in summer, when flows were greater and therefore more likely to cause a dilution effect. The apparent seasonal variation recorded from the two surveys, is in contrast to the data collected for Brandon Pithouse Acid Stream (see Table 5.1) and also the findings of Bennett (1969), both of which did not show seasonal variation. As mentioned in 4.3, the decreased levels of Na, Mg, Ca, Mn and H^+ were most likely due to dilution. The concentration of Mg, Ca and Mn ions seemed to be associated, since analysis of the data showed that there was a significant correlation between the elements on one or both surveys and also for Brandon Pithouse Acid Stream data (see Tables 4/12, 4/13 and 5.4).

The explanation for the increased concentration of some parameters is not clear, but it may be that during winter, the natural water table is higher in the mines and thus more of the substratum containing these ions is exposed to the acid water. This would lead to an increase of the elements in solution rather than a decrease due to dilution. Furthermore, it is likely that less sulphuric acid would be produced, because the increased level of water in the mine would reduce the amount of pyrites, which was available for oxidation by air, to produce the acid (see 1.22). This would account for the occurrence of higher pH values in winter and lower values in summer.

The higher acidity values in winter suggested that acidity was independent of H^+ concentration and that the H^+ only contributed some part of the acidity values measured. This suggestion was supported by the lack of significant correlation between pH and acidity in winter and a significant correlation in summer (see 4.7). There was also a stronger correlation between acidity and associated ions Fe, Al and SO_4-S, in winter than in summer.

The relationship between the ionic concentration and dilution water, must be dependent to some extent on the source of the acid water and whilst the system proposed above may occur where the water table is fluctuating, it may not be applicable to more constant sources, such as those originating from springs. As can be seen from the Brandon Pithouse Acid Stream source (Table 5.1), where the total discharge was relatively stable throughout the year, there was no marked seasonal variation in the chemical composition of the water.

8.25 Relationship between chemical constituents in acid water

Although it was difficult to determine whether the parameters measured were independent of each other, the statistical analysis suggested that some elements were associated with each other. From both the main surveys and data collected for Brandon Pithouse Acid Stream, it was shown that many parameters varied inversely with pH and directly with acidity. There also appeared to be an association between Fe, Al and SO_4-S concentrations, as these were significantly correlated at 95% level in both surveys and the Brandon Pithouse Acid Stream data.

8.26 Comparison with literature

Although there are relatively few detailed analyses of water below pH 3.0, nevertheless the examples in the literature are sufficient to allow comparison to be made with the data recorded in England. Several examples of chemical parameters measured by different authors are given in Table 8.1. Even though the levels reported by other authors tended to be lower, the water chemistry of the streams showed similar characteristics to the English sites. It would appear that the general characteristics discussed in 8.21 for the results collected in this study, are similar for most highly acidic mine waters i.e. high levels of acidity, Mn, Al, SO_4, Si and some heavy metals. All authors quoted in Table 8.1 agreed that levels of Pb are low compared with other heavy metals such as Zn.

Lower pH values than those reported in Table 8.1 have been recorded by other authors, for example, Fott (1956) measured water at pH 1.0 and several workers have reported values between 0.9 and 1.4 for the acidic Japanese lakes (Satake and Saijo, 1974). The data from the Japanese volcanic lakes

	conductivity micromhos	pH	acidity $CaCO_3$	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Co	Ni	PO_4P	NH_4N	NO_3N	SO_4S	Cl	Si
Hargreaves (1973), England.	30000	3.0	64000	2430	556	193	16.0	544	23000	3130	1.90	20.0	50.0	76	10.8	4.00	8600	255	114
$\bar{x}$ of 15 sites	600	1.5	110	488	4.0	0.09	0.006	0.35	7.5	3.72	0.001	0.01	0.05	0.01	0.05	0.15	250	14.0	5.9
Fuge (1972), Wales.		3.0		75	82	210	0.30	6.22	31.5	53							1680	5.1	14.8
1 site		2.75		13.5	45.0	23.7	0.10	2.52	3.0	6.0							261	5.0	6.5
Van Everdingen (1970), Canada.																			
(British Columbia)	3940	3.1		65.4	241	177	0.02	0.78	440	7.2	1.20						2138	0.4	16.7
$\bar{x}$ of 5 sites	1330	2.5		34.8	109	32.0	0.008	0.22	83.0	1.9	0.15						619	0.1	10.8
Hawley& Shikaze (1971), Canada																			
(Ontario)		2.0	14600	106	416	11.3	3.6	5.6	3200	588	0.67	3.8	3.2	5.0			7440		
Hancock (1973), South Africa.	3600	3.0		45	37				696						14	8	2510	780	
number of samples not given.															0.1	0.1			
Campbell et al. (1964), U.S.A																			
(Missouri)	1450	2.9	1756	232	308	67.0		83.0	6200					0.01			7600		92.0
2 samples	1200	2.0		31.5	63.0	6.5		3.9	104					0			777		5.2
Parsons (1964), U.S.A																			
(Illinois)		3.0	5300	285	410	21	3.0		296	183									
$\bar{x}$ of 7 sites		2.5	430	129	315	8.0	0.5		1.0	96									
Bennett (1969), U.S.A																			
(West Virginia)		3.1	1860		169				162					3.9		1.75			
$\bar{x}$ of 7 sites		2.6	313		73				49					1.0		0.33			
Patrick (1974), U.S.A																			
(Philadelphia)																			
number of samples not given,	775	3.0	64	14	48	0.10	0.10	1.76	21.5		0.08			0.06	0.24	0.32	320	10	4.5

All concentrations in mg l^{-1}

have not been included in Table 8.1 due to insufficient information, however, there does appear to be differences in the chemistry of these volcanic lakes, compared with the acid mine waters. In particular, the levels of iron reported by Satake and Saijo (1974) were low, although concentrations of SO_4-S were high. Presumably, this is a reflection of the source of the sulphuric acid, which, in the volcanic lakes, originates mainly from sulphur dioxide, rather than from pyrites.

The chemistry of the acid waters in England did not always correspond to the classification of acid water as proposed by Lundgren *et al*. (1971) and Parson (1964) (see 1.3). The main deviation from both classifications was for the expected iron and acidity values, at the corresponding pH value. For example, several sites had pH values below 3.0, but did not have acidity values of 1000.

8.3 Photosynthetic organisms present in acid waters

8.31 Geographical distribution and availability of inoculum

There was no geographical pattern for the distribution of species in the acid streams in England. Although no two streams were the same, similar species compositions were recorded in the S.W. England (site 14) as were found in the northern sites (eg. sites 1, 2, 3).

The widespread occurrence of most of the species and the ability of some to colonize rapidly newly formed acid waters (as seen at site 10 for *Euglena mutabilis*, see 7.2), suggests that the species are widely distributed in the aquatic habitat in general, and are available to occupy a suitable habitat when it arises. Both Lackey (1939) and Bennett (1969) made similar observations, although Bennett suggested that the acid stream organisms were more common in other habitats than Lackey and other workers had implied. There does seem to be some indication that the older, and perhaps more stable habitats (eg. sites 3 and 11), possessed the richer floras (see Table 4.7), whereas the younger acid sites (eg. sites 10 and 13) had considerably fewer species present. It is difficult to decide whether the larger floras are due to greater opportunity for colonization, over a longer period of time, or whether the apparent stability of the older sites (see 3.23) provides a habitat which has allowed less competitive species to become established, as well as the more resistant ones. Lackey (1939) reported that few new species established themselves after the initial inoculation of a new acid site had occurred. However, he does not indicate the time necessary for the stabilization of a community to occur. An investigation into the occurrence of acid tolerant species in the immediate catchment area of an acid stream (Pomfret, 1973) showed that most species found below pH 3.0 were also represented at high pH values. It was also noted that the more acid tolerant species, especially *Euglena mutabilis*, were not present in such large numbers. This observation was made also by Bennett (1969) and Weaver & Nash (1968).

8.32 Frequency of occurrence

The results of the surveys of streams in England and the low pH reaches of Brandon Pithouse Acid Stream, indicate that the extreme acidic environment is characterized by relatively few photosynthetic organisms. Of the 28 species recorded at and below pH 3.0, in waters associated with mining, only 8 of these were recorded in 20% of the reaches and 5 species in 10% of all reaches (see Table 4.4). Euglena mutabilis, Pinnularia acoricola and Gloeochrysis turfosa were the most widespread and abundant, whilst Nitzschia subcapitellata Nitzschia elliptica var. alexandrina, Eunotia exigua and Chlamydomonas applanata var. acidophila were widespread but not very abundant. The filamentous species Hormidium rivulare and Zygogonium ericetorum were invariably very abundant but only moderately widespread (see Tables 4.4 and 4.8). The fore mentioned species not only represented the main algal population of the lower pH reaches of Brandon Pithouse Acid Stream, (Table 5.6), but also at times represented species which occurred in the less acid reaches of that stream (Table 5.6, reaches 7b, 8 to 11).

As mentioned in 4.41, the protonema of both Dicranella sp. and Drepanocladus fluitans was more widespread than the adult form. The occurrence of the protonema appears to be more common in the acid habitat then might otherwise be expected. The reasons for the protonemal dominance are not known, but it was concluded that pH alone was not responsible for regressing the transition from protonema to adult (see 7/26).

8.33 Relative abundance

As mentioned in 1.51, the occurrence of large numbers of individuals of one species is a characteristic of highly acidic waters. The data presented in 4.45 and 5.8 are in agreement with Lackey (1939), Joseph (1953) and Bennett (1969), all of whom reported that although only a limited number of algae were present in acid streams, they were invariably present in large numbers. Lackey estimated that *Euglena mutabilis* was often in concentrations of over 10^6 cells mm^{-2}. On several occasions, estimations were made of the density of *Euglena mutabilis* near the source of Brandon Pithouse Acid Stream A. These estimations showed that at times, there were twice the number of cells recorded by Lackey.

Algal species which were frequently macroscopically obvious included, *Euglena mutabilis*, the diatom species, *Hormidium rivulare* and *Zygogonium ericetorum*, as well as the protonemal stages of both moss species (see 4.44). Although the methods used for determining the abundance of one species relative to others, were susceptible to many user errors (see 2.53), the technique nevertheless provided more information about a species than would have been gained by merely recording presence and absence. Relative abundance values gave some indication as to whether a species could just tolerate certain conditions, or whether it could thrive in that environment. Both Lackey (1939) and Bennett (1969) for their studies on algae and more recently Herrick and Cairns (1971) and Dills and Rogers (1974) both working with macroinvertebrates, found that abundance values revealed considerably more about the environment than merely the knowledge of presence or absence. The practice of adding the maximum abundance values, as used in this study for determining the seasonal cycles (see 5/10), is technically unsound. However, whilst accepting the incorrect usage and the fact that one does not know the individual scores which gave the final value for each season, the technique did provide some information which would not otherwise have been obtained.

8.34 Maximum standing crop values for Brandon Pithouse Acid Stream

The maximum standing crop values recorded for Brandon Pithouse Acid Stream demonstrated the decrease in crop with an increase in pH value (see 5.81). Several of the more dramatic decreases could be accounted for by precipitation of ferric hydroxide (eg. reach 13) and to a lesser extent changes in topography and flow characteristics (see 5.8).

The fluctuations in M.S.C. values at the individual reaches were due to some extent, to seasonal variation in growth rates of the algae (see 5/18). Physical factors such as scouring by run-off and large changes in pH regime, as occurred at reach 8, (see 5.84) were also responsible for some of the variations in values. As previously mentioned, maximum values were obtained at reach 1 were due to extensive growths of Euglena mutabilis. The variations in M.S.C. value at this reach were mainly due to seasonal variation in the growth of this species (see Fig. 5/29a), as the reach was characterized by a relatively stable current speeds and a stable substratum as well as a stable chemical environment. Some of the fluctuations were most likely due to grazing by herbivorous animals. Brook (1955) attributed the seasonal distribution of algae, which could not be invoked by abiotic factors, to extensive grazing, whereas Moss (1968) suggested that stability of substratum on which the benthic algae lived was the most important factor relating to algal crop size. It would seem from these results that this was also partly the case for the acidic environment. Further discussion of factors influencing the algal crops have been included in 8.9.

8.35 Comparison with values recorded for other habitats

A comparison of the maximum standing crop values recorded for Brandon Pithouse Acid Stream, with those given by Moss (1968) and Marker (1976a) show that the values for Brandon Pithouse Acid Stream, reach 1, were larger than those recorded elsewhere. The largest value reported by Moss was 2.35 $\mu g\ mm^{-2}$ for epiphytic algae and Brock & Brock (1966) reported values of 0.8 $\mu g\ mm^{-2}$ for non acidic thermal springs. The latter value is considerably less than the values recorded here (3.2 $\mu g\ mm^{-2}$). The values have been expressed here as $\mu g\ mm^{-2}$ rather than $\mu g\ m^{-2}$ for the purpose of comparison.

It is possible that some of the differences between values mentioned above, are due to the use of different harvesting techniques. Although the method adopted here was subject to error, it was considered that the error was sufficiently small as to enable meaningful comparisons to be made between sites. Harvesting of the streams substratum was used in preference to the implementation of artificial substrata because of the possibility of selecting specific organisms by the use of the artificial method. Nevertheless, even allowing for a large degree of error, the values recorded remain compatible with the values given by Moss (1968). This implies that productivity in acid streams dominated by a few species, is likely to be high compared with other habitats. However, the work of Stickney and Campbell (1972) did not support the results recorded here, they estimated the rate of production of accrual *Aufwuchs* on glass slides in strip-mine lakes and found that the rate was significantly greater in alkaline than in the acid waters

8.36 Seasonal variation

The data both from Brandon Pithouse Acid Stream and the two general surveys, indicated that the flora of acid streams is relatively stable throughout the year. As mentioned in 4.42, only two species were recorded in late winter which were not recorded in late summer and the converse was found for four other species. Apart from *Lepocinclis ovum*, all the other five species mentioned above were recorded only on one occasion.

The data from Brandon Pithouse Acid Stream, where samples were taken throughout the year (see 5.8 and 5/10) indicated that the fluctuations in abundance and maximum standing crop were almost entirely due to seasonal variation and physical factors such as scouring, rather than the introduction of different species. Lackey (1939) found stable populations in the streams he studied, during summer and winter, whereas Bennett (1969) reported that there were quite large differences in populations throughout the year. The results from this study support Lackey's findings rather than those reported by Bennett.

8.37 Comparison with available literature

Although some comparisons with the available literature have been made for individual species in chapter 7, an overall comparison with the data from the U.S.A. has been included in this section. As the exact pH values for the species recorded in Bennett's (1969) account were not given, the occurrences listed for Bennett (Table 7.2) are assessed on abundance in 'acid creeks', of which six out of eight were below pH 3.0. Several other authors from the U.S.A. have been omitted because of the lack of data for pH values for the species. Data from Japan and South Africa have also been omitted for the same reasons, however, where relevant, individual values have been included in the text.

Of the 40 species listed in Table 8.2 as growing below pH 3.0, only four were common to both England and the U.S.A. These include Euglena mutabilis, Lepocinclis ovum, Eunotia exigua and Ulothrix zonata. Two of these, Lepocinclis ovum and Ulothrix zonata were not very widespread below pH 3.0 in England.

Table 8.2

Comparison of algal species at or below pH 3·0 in U.S.A. and England. L, Lackey (1938); S, Steinback (1966); W, Warner (1968); B, Bennett (1969)

Species	U.S.A. L	S	W	B	England 1973-74
Chlamydomonas applanata var. *acidophila*					+
Chlamydomonas globosa				+	
Chlamydomonas spp.	+	+		+	+
Cryptomonas erosa	+				
C. ovata				+	
Cryptomonas sp. (not above)					+
Chromulina ovalis	+			+	
Characium sp.					+
Chlorogonium elongatum				+	
Desmidium sp.	+				
Euglena mutabilis	+	+	+	+	+
Euglena sp.				+	
Eunotia exigua		+	+	+	+
Frustulia rhomboides				+	
Gloeochrysis turfosa					+
Hormidium rivulare					+
Lepocinclis ovum	+				+
Microthamnion strictissimum					+
Mougeotia sp.	+			+	
Navicula spp.	+	+			+
N. nivalis					+
Nitzschia subcapitellata					+
N. elliptica var. *alexandrina*					+
N. palea					+
N. ovalis					+
Nitzschia spp. (not above)					+
Ochromonas sp.	+				
Penium jenneri				+	
Phaeothamnion sp.	+				
Pinnularia acoricola					+
P. braunii				+	
P. microstauron					+
P. termitina			+		
Stichococcus bacillaris					+
Synedra rumpens				+	
Ulothrix zonata	+				+
U. subtilis				+	
U. tenerrima			+	+	
Ulothrix sp.				+	
Zygogonium ericetorum					+

As mentioned in 1.62 there were discrepancies between the different American studies, as to which species were the most resistant and widespread. Euglena mutabilis, Eunotia exigua and various species of Pinnularia were common to all surveys and the diatoms have also been reported in South Africa by Harrison (1958) and Hancock (1973), and in Japan by Uéno (1958). Species of Ulothrix and Chlamydomonas were also recorded by most workers studying algae in acid streams. Species that were found to be widespread in England, but were not reported present below pH 3.0 in the U.S.A. were: Nitzschia spp., Pinnularia acoricola, Hormidium rivulare, Gloeochrysis turfosa, Zygogonium ericetorum. Records of Pinnularia acoricola have been made in South Africa (Hancock, 1973) and Zygogonium ericetorum has been reported above pH 3.0 in the U.S.A. (Weaver & Nash, 1968). Although species of Hormidium have been reported above pH 3.0 (Weaver & Nash, 1968) no records of Hormidium rivulare were found in the literature for low pH (see 7/10).

It is likely that because of the large morphological variation displayed by some organisms, some of the species listed as different in Table 8.2 are, in fact, the same species. For instance, the Chlamydomonas, Ulothrix and Pinnularia species may well be the same species in some cases. It is interesting that Lackey (1939) was the only author to report the presence of protonema, when in England it was both widespread and abundant.

The absence of Cyanophyta from the streams in England agrees with previous observations by Brock (1973) and other authors (see 1.62). In Brandon Pithouse Acid Stream, Oscillatoria pseudogeminata was recorded at pH 5.8 but was not recorded when the pH value decreased below pH 5.0. In contrast to other studies, Bennett (1969) reports the presence of Phormidium retzii and Oscillatoria agardhii in acid creeks. Because he does not quote pH values for the species it is impossible to determine whether they were present below pH 4.0.

As previously mentioned in 1.62, a comparison of the total number of species recorded in the acid habitats shows that Bennett's results differ from those of other workers. He recorded 107 genera between pH 2.6 and 4.1 at 8 stations, whereas Lackey (1938) reported only 18 from 63 stations; Weaver and Nash (1968) reported 20species from 6 stations on one stream, and 23 algal species were recorded from 54 different 10 m reaches, at 15 sites in the English acid streams.

These apparent differences in the size and composition of the floras suggest that, apart from a few species which are likely to be found in acid streams anywhere in the world (Euglena mutabilis and Eunotia exigua), the majority of the floras reflect the indigenous population of other habitats besides the acid environment. In addition to the influence of the geographical distribution of the species, it is probable that the floras are a reflection of the amount, the stability and the age of the acidic water present in any given area. In areas where a reasonably large proportion of the surface water is of low pH, it is likely that because a greater number of organisms will have been subjected to the acid conditions, a greater number of resistant species will appear.

The occurrence of *Euglena mutabilis* and *Eunotia exigua* in most reports on acid waters, implies that they have a worldwide distribution and are, therefore, available to colonize acid sites where they occur. In contrast, the apparent absences in the U.S.A. and elsewhere of *Pinnularia acoricola* and *Hormidium rivulare*, two species which were especially common in England, suggest that their distribution is geographically restricted. Until more is known of the distribution of these species in non acidic habitats, it will be difficult to determine, with any certainty, the relative importance of the indigenous population when considering the formation of the flora of acidic waters.

8.4 Factors affecting distribution of photosynthetic species

The complete absence of a taxonomic group, ie. the Cyanophyta, from waters below pH 3.0 categorizes it as an extreme environment (Brock, 1969). Whilst all the species represented below pH 3.0 must be considered resistant to large concentrations of hydrogen ions and in most cases high levels of heavy metals, (see 4.51) it is not clear which factors have a controlling influence on the occurrence and abundance of the algae. Furthermore, it is clear from the results that some species are more tolerant to acidic mine waters than others (Tables 4/10 and 5.6)

8.41 Relationship between the number of species and pH in the field

The pH of the water is commonly used as the criterion for indicating the tolerance of species to the acidic habitat and has long been recognized as a factor which may limit their distribution (Bennett, 1969). Whitford (1959) suggested that while recognizing that pH alone cannot strictly indicate the type of flora, it is still the best and most readily available method for indicating an ecotype. One of the problems associated with the extreme acid environment, is that pH influences directly the concentration of the ions in solution through increased solubility and therefore, as already discussed, it is difficult to dissociate the effect of one ion from another.

The results showed that, in general, the number of species increased with an increase in pH and that for many of the species recorded, there was a pH growth limit above pH 2.5. (see 4.51, 5.6 and chapter 7). However, statistical analysis of the data for the two surveys and Brandon Pithouse Acid Stream were not in agreement. For instance, there was no significant correlation at the 95% confidence limit between species and pH in the late winter survey (Table 4/13), whereas there was a significant correlation at the same level in summer (Table 4/12) and over the pH range 2.6 to 8.0 down Brandon Pithouse Acid Stream (Table 5.4). As previously mentioned, there was also a significant correlation (p = 0.001) between pH and the maximum standing crop (Table 5.9). The discrepancy between the results suggests that below pH 3.0, factors other than pH are influencing the distribution of species, whereas over the wider pH range in Brandon Pithouse Acid Stream, pH was the main controlling factor influencing distribution of the species.

As mentioned in 5.75 the data collected for Brandon Pithouse Acid Stream were sufficient to allow meaningful stepwise regression analysis to be performed. The results of this analysis supported the implication made by linear correlation analysis for Brandon Pithouse Acid Stream and out of the twenty four parameters tested, pH was found to exert the greatest influence on the number of species (Table 5.8). It is possible that the additional sites, below pH 3.0, would help to elucidate the discrepancy between the results for the two general surveys.

8.42 pH as a growth limiting factor

Both the field and experimental results suggest that pH alone does limit growth of algae. For instance, although only 15 out of 95 reaches had pH values of less than 2.5, (see 4.52), species which were widespread but were never recorded within this range included Nitzschia subcapitellata Eunotia exigua, Hormidium rivulare and Zygogonium ericetorum. As mentioned in chapter 7, examples occurred of individual streams where the organism was absent at lower pH values but was recorded in adjacent reaches at higher pH values.

The experimental data for the five species of algae (see 6.31) showed that all species had an optimum growth pH range and a pH growth limit. A comparison of these values with those recorded in the field, showed quite good similarity (Table 6.3), thus suggesting that even though other factors were present in the field, the main controlling parameter was pH.

8.43 Comparison with available literature pertaining to pH

Although other authors have not considered the large number of parameters measured in this study, several have considered a few parameters and found pH to be the main factor influencing growth. Steinback (1966) reported a significant correlation between pH and the number of algal species and Lackey (1939), Bennett (1969) and Stickney & Campbell (1972) found that species number increased with an increase in pH. Dills & Rogers (1974) applied statistical tests similar to those applied here and found that for macroinvertebrates, growing within the range pH 2.8 to 6.2 H^+ concentration was highly correlated with species number. Such correlations, however, do not necessarily imply a causal relationship although it may be indicative of such a relationship.

8.44 Effect of acidity on distribution and growth of species

Bennett (1969) reported that although pH was a good indicator of the ecotype flora, acidity seemed to have the controlling influence on the algal flora. Unfortunately, few other authors have considered this parameter with regard to the number of species. As mentioned previously in the discussion, acidity and pH are not necessarily related because of the presence of acid salts. The data collected here support, to some extent, Bennett's findings, in that statistical analysis of the data for the winter survey and Brandon Pithouse Acid Stream showed a significant negative correlation between acidity and species number, at the 95% confidence limit. However, no such correlation was recorded between the two parameters for the summer surveys and neither did the stepwise regression analysis for Brandon Pithouse Acid Stream data show acidity to be closely related to species number (Table 5.6).

Several other factors suggested that acidity was not the main influence on growth of the algae. For example, with the exception of a few very high acidity values, there was no obvious acidity value above which species were restricted, as was apparent at pH 2.5. If acidity was the main factor involved, then it is unlikely that such good similarity would have been recorded for pH between field and experimental results, as mentioned in 8.52. Furthermore, the acidity values at pH 2.5 in the medium which was acidified with sulphuric acid, were considerably less than those recorded at pH 2.5 in the field (eg. in field maximum value at pH 2.6 was 20000 mg l^{-1} $CaCO_3$, whereas in the medium the value was approximately 1000 mg l^{-1} $CaCO_3$).

It would appear, therefore, from these results, that it is unlikely that acidity had a greater influence on species number than hydrogen ion concentration.

8.45 Effect of heavy metals on distribution of species

Other chemical factors likely to affect growth were the large concentrations of heavy metals present in the water. Whilst all the heavy metals, except perhaps Pb, were at times very high compared with unpolluted surface waters, the analysis of data did not indicate that they were the main factor limiting growth in the extreme acidic environment (see Tables 4/12, 4/13). The data from the experiments carried out on a population of Hormidium rivulare and Euglena mutabilis with Zn, show that the growth of both species was limited by high concentrations over a range of pH values (see 6.4). However, the toxicity of the metal was found to vary with the pH.

8.46 Relationship between pH, Ca and Zn toxicity

The toxicity of Zn increased with an increase in pH and both organisms tested were most resistant to Zn at their optimum growth pH ranges (see 6.31, 6.41 and 6.42). Similar results were obtained between the concentration of Cu and a population of Hormidium rivulare (6.43).

As mentioned in 6.61 and 6.63, experiments also demonstrated that the tolerance of H. rivulare to Zn could be antagonized by the addition of Ca, whereas the tolerance to pH was not improved by the addition of this element. As the toxicity of Zn was antagonized by Ca and pH, it would seem unlikely that Zn concentration controlled the growth of the algae at low pH. Whether other heavy metals present in acid mine water behave in a similar fashion to Zn is not known. The experiment with Cu suggests that pH may affect the toxicity of other heavy metals in much the same way.

Whilst the heavy metals may not exert overall control on species numbers, the experimental data on the growth of *H. rivulare* suggest that where a species is near the pH growth limit, the presence of moderately large concentrations of Zn and probably other heavy metals, may provide sufficient additional stress on the organism to cause a further reduction in growth. This may eventually lead to the death of the whole population. This was evident at pH 2.5 and 2.75 for concentrations of Zn between 5 and 10 mg l^{-1} (Fig. 6.4). Conversely, at growth limiting concentrations of heavy metals, a reduction in pH to values near the pH limit, would probably have a similar effect, even though the toxicity of the metal was reduced by a decrease in pH.

8.47 Comparison with available literature pertaining to heavy metals in the acidic environment

Other workers in this field have not attempted to dissociate the effect of pH and heavy metals on organisms growing in acid mine waters. As mentioned in 1.72, Droop (1974) suggested in the absence of experimental data, that intolerance shown by many algae to low pH was due to the high level of heavy metals. Besch et al. (1972) found from field studies on diatoms in acidic and non acidic, Zn and Cu polluted waters, that acid tolerant species were also tolerant to high heavy metal levels, but that species tolerant to heavy metals were not tolerant to low pH. From their observations they suggested that pH was probably the primary factor influencing species distribution, although metals were selective to some extent at low pH and more markedly so where extreme acidity was absent. The data collected for the four populations of *Hormidium rivulare* and the populations of *H. flaccidum* and *H. scopulinum* (Fig. 6.7 and Table 6.6) supported the observations made by Besch *et al.*, in that although *H. flaccidum* and *H. scopulinum* were tolerant to quite large concentrations of Zn, they would not grow below pH 4.0, whereas populations of *H. rivulare* were tolerant of low pH and in some cases large concentrations of Zn (see 6.51).

8.48 Requirements of *Hormidium rivulare* and *Euglena mutabilis* for levels of Al and Zn

The experimental data on the effects of increased levels of Al on *Hormidium rivulare* (6.62) and Zn on *Euglena mutabilis* (6.42) demonstrate that both organisms had a requirement for low levels of these elements. As mentioned in 1.71 a similar response to Al concentration was shown by Foy & Gerloff (1972) for a strain of *Chlorella pyrenoidosa*. This strain grew in 48 mg l^{-1} Al at pH 4.6 and gave a positive growth response to concentrations between 1.5 and 12 mg l^{-1} Al. Similar concentrations were also found to stimulate the growth of *Hormidium rivulare*.

8.49 Effect of nutrient levels on the number of species

Apart from a few PO_4-P values, the only ion which was recorded at a level sufficiently low as to suggest that it might seriously hinder plant growth was K, which on one occasion was as low as 0.05 mg l^{-1} (see 4.15).

The stepwise regression analysis on Brandon Pithouse Acid Stream data, indicated that nitrate and phosphate concentrations were relatively important in determining the number of species present. However, similar relationships were not found in the general surveys. As previously mentioned in 2.72 the addition of NH_4-N to the medium improved the growth rate of *Gloeochrysis turfosa* and *Chlamydomonas applanata* var. *acidophila*. The analysis of field data did not indicate that NH_4-N was very relevant in determining the number of species, although there was significant correlation between NH_4-N and number of species in the summer survey. It may be that some organisms utilise NH_4-N in preference to NO_3-N and that in the field there was sufficient NH_4-N (mean 2.20 mg l^{-1} NH_4-N) to allow a good growth rate, without using NO_3-N.

8.5 Effects of some elements on the growth of *Hormidium rivulare*

8.51 Requirements

It was shown that *Hormidium rivulare* had a requirement for low levels of Ca, Mg, Al and PO_4-P at pH values between

2.5 and 6.0 (6.6). As levels recorded in the field for these elements, rarely fell as low as those in 6.6, it was assumed that they would not have restricted growth to any great extent.

The various requirements for Ca, Mg and PO_4-P by algae, are widely accepted and levels have been determined for several species (Gerloff & Fishbeck, 1969; Hutner et al., 1950). However, it would appear that the levels required in the low pH environment were greater than at higher pH values. Macias (1965) reported that the requirement for Chlamydomonas mundana for Na, Ca, Mn, and Fe were greater at low pH. The reasons for the increased requirements are not understood, but it is possible that at low pH the elements may alter their ionic state and become less available for uptake by the organisms.

8.52 Effects of large concentrations of Ca, Mg, Al

With the possible exception of PO_4-P, all elements tested antagonized growth at the highest concentration examined. The reasons for the observed decrease in growth rate are not understood, but it is possible that the ions may be competing with other essential elements for uptake and that these other elements may become limiting at the optimum pH growth range. However, levels as high as those which caused antagonism in the growth rate, did not appear to have an adverse effect on field populations. For instance, the mean Ca level recorded for Hormidium rivulare was 287 mg l^{-1} a value higher than the level which antagonized growth in the laboratory.

8.6 Effect of Fe and physical parameters on the distribution of species

8.61 Fe

The large concentrations of Fe often associated with highly acidic mine waters are considered by some workers to be as important in controlling the growth of organisms as the effect of pH (Hynes, 1970). However, the main effect the Fe has on the environment is due to the precipitation of ferric hydroxide rather than one of toxicity, although the latter has been reported for some plants (Oborn, 1960). The addition of Fe to the culture medium did not cause a reduction in growth rate at low pH (see 6.32).

As mentioned in chapter 5, the continuous precipitation of iron, as seen at reaches 8 and 13 of Brandon Pithouse Acid Stream, resulted in a decrease in the number of species (5.64) and the standing crop of algae (5.86). The reasons for the observed effects on the algae, were considered to be due to a reduction in light reaching the bed of the stream and the formation of an unstable substratum, both of which were most obvious at reach 13. Where the iron precipitate did not form a continuous blanket, as seen at reach 8, then the reduction in standing crop was slightly greater than the decrease in number of species, whereas the more flocculent precipitate broughout about by the formation of Fe and Al complexes, caused a marked reduction in both number of species and standing crop. (see Fig. 5/28, reach 13).

8.62 Experimental data pertaining to Fe

The reason for the decrease in growth rate of five species of algae (see 6.32) in the presence of a small amount of ferric hydroxide, is not clear. Analysis of the media containing stream water, after the ferric hydroxide had precipitated above pH 3.0 (see Table 6.2), showed that the level of Fe remained sufficiently high as to improve the growth of Chlamydomonas acidophila, as reported by Cassin (1974). Based on Cassin's results it was assumed that 1.40 mg l^{-1} Fe would not limit growth of the test algae and therefore it seems probable that the concentration of some essential trace element was reduced in the medium. The possibility that at the higher pH values, the levels of heavy metals introduced to the media from the stream water may have become toxic, is very unlikely. The same population of Hormidium rivulare as used in the experiment under discussion, was shown to tolerate much higher concentrations of Zn and Cu at pH values above 3.0, than were present in the media after precipitation (see Table 6.2). It is possible that the metals acting additively or synergistically, could have caused the decrease in growth rate, but it seems unlikely particularly in view of the maximum resistance shown by the test organism to Zn and Cu, between pH 3.0 - 4.0. The amount of ferric hydroxide which precipitated out of solution above pH 3.0 (11.7 mg l^{-1} Fe), was insufficient to have caused a significant reduction in growth rate due to the reduced light penetration.

8.63 The influence of temperature on growth

There was no indication from the experimental results that temperature was a major influence on presence of a species, although over the temperature range recorded in the field, it is likely that the growth rate was affected (see 6.7) on a seasonal basis. These results also demonstrate that for these species there is no thermophilic resistance, as demonstrated by some species in the thermal acid streams.

8.64 Effect of other physical factors on distribution and abundance

Other environmental factors which affected the biomass of the algae and to a lesser extent the number of species, include the stability of the substratum, the scouring effects of fast current speeds, predation and the competitive nature of the algae.

The maximum standing crop results (see 5.82) serve to illustrate the effects of physical factors on the algae in an acid stream. Although it is likely that these factors may, on their own, be responsible for the presence or absence of a species, results indicate that their contribution was mainly in controlling the numbers of individuals of a species.

As mentioned briefly in 8.34 the effect of an unstable substratum and the scouring effect of fast current velocity, probably account for a large proportion of the differences between standing crop results for Brandon Pithouse Acid Stream, reach 1, and other downstream reaches. As referred to in 5.82, the first 10 m of Brandon Pithouse Acid Stream were not subjected to intermittent high flows due to run-off Consequently, the density of algal material was allowed to build up without being removed by washout from flood waters. Even though the substratum in Brandon Pithouse Acid Stream was relatively stable, compared with some acid streams, the increased current speeds caused by flood waters were sufficient to remove the algal material growing on the top layer of clay. The field results also suggest that stability of the substratum is probably more important than its composition (see 3.22).

As previously mentioned, the current speed tended to select species. For instance, as mentioned in 7.9 and 7/13 the permanently flagellated species Lepocinclis ovum and Chlamydomonas spp. were recorded mainly from reaches of the lowest current speeds (see Table 4.9). However, Chlamydomonas spp. were present at faster speeds, but in association with larger organisms such as the mosses. Moore & Clarkson (1967) studied a few of the physical and chemical factors influencing the growth of vascular plants in acidic water (mostly greater than pH 3.0) and concluded that substrate stability and type were the most important factors which determined the distribution of plants.

8.65 Habitat type

Although the individual habitat preferences of each species have been dealt with in chapter 7, further general observations are considered here. The observations discussed in 8.64 suggest that habitat type is important for a few species, in particular, those which are permanently motile and those which are easily removed by the action of fast current speeds.

The data presented in 4.43 does not indicate that habitat was very important for frequently occurring species; however, a comparison of the abundance values (see Table 4.7) for some species indicates that although they may be present in a particular habitat, they are more abundant in another. For example, Chlamydomonas applanata var. acidophila was present in faster current speeds, but was more widespread and more abundant in the pool habitat (see Table 4.7). In contrast, Hormidium rivulare tended to be most abundant in the stream habitat. The motile stage of Gloeochrysis turfosa and the flowering plants, was recorded more frequently in the pool habitat.

8.66 Predators and interspecific competition

Herbivorous predators are known to reduce the standing crop of algae in unpolluted streams (Moss, 1968), therefore, because acid tolerant animals have been recorded (1.5), it is likely that these organisms will have some effect on the standing crop of algae in these acid streams. Although no quantitative or qualative samples were taken of the animal populations, two species of rotifer were regularly observed in samples taken from Brandon Pithouse Acid Stream. It was obvious from the gut content of the animals that they were grazing on algae and at certain times of the year (usually spring-summer) they were in sufficient numbers as to reduce the standing crop. However, until a comparison of the numbers and grazing capacity of the rotifers is carried out, their influence must remain speculative.

It is likely that adaptation to extreme low pH environments results in a lowering of the competitive ability of the t lerant species when they are not in the presence of low pH. There was some indication that Euglena mutabilis displayed a reduction in it's competitive ability at pH values above 3.0. For example, although reach 1 and reach 10, Brandon Pithouse Acid Stream, supported almost the same number of species (see Table 5.6), reach 1 (pH 2.6) was dominated throughout the year by Euglena mutabilis, whereas it was never abundant at reach 10 (pH 3.2). As experimental results (Fig. 6.3) did not indicate that growth was reduced at pH 3.0, it is possible that the difference in abundance of E. mutabilis was due to a reduction in it's ability to compete with other species (eg. Hormidium rivulare) at pH values greater than 3.0. This suggestion was supported by Lackey (1939) who found the alga to be infrequent and rarely abundant in the presence of other species, at higher pH values.

8.67 Resumé of factors influencing the growth of photosynthetic organisms of low pH

It is clear from the results that many factors, both physical and chemical, are likely to influence the presence and abundance of species in highly acidic waters. However, the data do suggest that of these factors, pH has the major influence on the presence of a species in a particular habitat, and that other factors are primarily concerned with influencing the abundance of an organism once it is established at a particular pH value. It is likely that acidity and heavy metals may act synergistically with pH, in providing additional stress so as to restrict the growth of a species at sites where pH alone would not.

8.7 Ordination of species on the basis of pH tolerance

If pH is considered to be the main factor controlling the distribution of species in acidic waters, then it is possible to ordinate those species which were recorded in the two surveys and also those which occurred in Brandon Pithouse Acid Stream, on the basis of their tolerance to pH. The results given in Table 5.7 divide the species recorded in Brandon Pithouse Acid Stream system into four groups (see 5.7). As can be seen, there was very little overlap between the Groups I and III on the basis of occurrence over the pH range examined.

It is possible that the species in Group II and III are tolerant to aspects of acid mine drainage other than low pH and that their competitive ability in the presence of moderately large concentrations of heavy metals and other ions present in the mine waters, is selective in their favour.

Table 8.3 includes the list of species recorded below pH 3.0 and shows the lowest pH values at which the organism was

Photosynthetic organisms in order of tolerance to pH (where possible laboratory results have been included).

species	lowest pH recorded in field	lowest pH recorded in laboratory
Euglena mutabilis	1.5	1.3
Pinnularia acoricola	1.5	
Characium sp.	1.5	
Gloeochrysis turfosa	1.8	1.5
Chlamydomonas applanata var. acidophila	1.8	1.3
Stichococcus bacillaris	1.8	1.5
Nitzschia subcapitellata	2.5	
Nitzschia elliptica var. alexandrina	2.5	
Eunotia exigua	2.5	
Hormidium rivulare	2.5	2.5
Zygogonium ericetorum	2.5	
Dicranella sp.(protonema)	2.5	
Lepocinclis ovum	2.5	
Nitzschia sp. typeA	2.5	
Nitzschia ovalis	2.5	
Pinnularia microstauron	2.5	
Navicula sp.	2.5	
Nitzschia sp. type B	2.5	
Typha latifolia	2.5	
Dicranella sp.	2.5	
Drepanocladus fluitans	2.6	
Cryptomonas sp.	2.6	
Drepanocladus fluitans (protonema)	2.6	
Chlamydomonas sp.	2.7	
Nitzschia palea	2.7	
Juncus effusus	2.7	
Micothamnion strictissimum	2.9	
Navicula nivalis	3.0	
Ulothrix zonata	3.0	

recorded, both in the field and, where applicable, in the laboratory. As mentioned earlier it is evident from these data that the majority (23 out of 27) of the species were not recorded below pH 2.5. Hormidium rivulare is an example of one of the more commonly occurring species which was not recorded below pH 2.5, either in the field or under laboratory conditions. Although more data are necessary to verify this observation, the lack of records for these species below pH 2.5, in this study suggests that there may be a "cut off" point at pH 2.5, below which many species will not grow. The classification system commonly used for separating species into different categories, depending on their pH tolerance, was proposed by Hustedt (1939). This classification includes all the species in Table 8.3 and many of those in Table 5.6 Group II, as acidobiontic species (ie. those species which occur at pH values below 7 with an optimum distribution at pH 5.5 or less). The distribution of algae shown in this study suggests that Hustedt's system could be extended to separate the extreme acidobiontic species which are capable of growth below pH 2.5, from those which are restricted to values above pH 2.5.

8.8 Morphology

Morphological variations were displayed by many of the species recorded, both under field and laboratory conditions. For the five species used in experiments (see 6.35, 6.41 and 6.42), the greatest degree of morphological variations were observed near the growth limiting pH values. Similar variation was not observed at the higher pH values, where in all cases, the growth rate was also reduced. As mentioned in 6.35 in the case of Stichococcus bacillaris, obvious morphological differences were not associated with a marked reduction in growth rate, except at the actual pH limit (see 7.15). Similarly, morphological differences were recorded for Eunotia exigua and Pinnularia acoricola in the field, at pH values which did not appear to restrict the growth rate of the algae, although in the case of Eunotia exigua the degree of malformation of the frustules was greater at lower pH values (see 7.8).

Similar differences in morphology were observed for Hormidium rivulare and to a much lesser degree for Euglena mutabilis, in the presence of growth limiting concentrations of Zn and Cu (see 6.4). It is not known whether similar differences were caused by large concentrations of heavy metals in the field. As mentioned on several occasions in chapter 7, these morphological variations caused taxonomic problems. It is possible that one species of Navicula and two species of Nitzschia were recorded here that have not been previously described (see 7/16, 7/17, 7/19). However, in view of the marked differences in form recorded for some species, it is possible that these specimens were merely variants of known species which displayed a different morphology in the extreme acidic environment (J.R. Carter, pers. comm.). If such variations are characteristic of species in the extreme acidic habitat, then it is likely that errors may have occurred in identification and that the differences in the species lists given in Table 8.2 may be artificially large. Without detailed descriptions of species it will be almost impossible to resolve this problem.

The process of cell malformation has not been studied to the author's knowledge; presumably the hydrogen and heavy metal ions affect some part of protein synthesis. The observations indicate that although the growth rate is reduced in most instances, the cells are still capable of division and if maintained in growth limiting conditions, the population becomes dominated by the malformed cells. On occasions when populations of _Hormidium rivulare_ and _Stichococcus bacillaris_ which were dominated by malformed cells, were transferred to media at their optimal growth pH, the number of malformed cells decreased and eventually disappeared, showing that the morphological changes were not a permanent feature of that population. However, whether they would do so after a longer period of time is not known.

8.9 Adaptation and possible mechanisms involved in acid tolerance in plants

It has already been mentioned in 1.73 that little information is available concerning the pre-adaptation and mechanisms necessary for an organism to survive in acid mine waters. The large differences in the species composition recorded for acid streams throughout the world, suggest that there are many species which have the necessary specialization to survive in this environment. However, it is not known whether this ability is inherent, or is due to physiological adaptation over a period of time. The results of both the field and laboratory results indicate that species are pre-adapted to the acidic environment, but that some physiological adaptation also takes place.

The pH growth curves of the five species isolated from Brandon Pithouse Acid Stream (see Table 6.4), showed that with the exception of Hormidium rivulare, all species grew at lower pH values than those from which they were isolated, with the lowest value approaching the lowest field value recorded anywhere in England (see 6.34). Likewise, the population of Hormidium rivulare and Euglena mutabilis showed that they were able to withstand considerably greater levels of Zn and Cu in the laboratory than the field. Further evidence is given by the fact that prolonged growth at pH 6.0 did not affect the pH resistance of Hormidium rivulare (see 6.33).

There does appear to be differences in tolerance which may be due to physiological adaptation. For example, the variation in the pH tolerance within the range pH 2.5-6.0 for different populations of H. rivulare (see Fig. 6.7). Also, the difference in tolerance to Zn concentration, as illustrated by a comparison of the maximum levels tolerated by one population isolated from Brandon Pithouse Acid Stream (max. 30 mg l^{-1} Zn) as compared with a field population at site 14 where it was growing abundantly at 67.4 mg l^{-1} Zn. In one experiment a population of H. rivulare was exposed to pH 2.5 for several months, however, this did not improve the growth rate at that pH value, nor did it increase tolerance to pH values below 2.5. This would suggest that any physiological adaptation that occurs either takes place over a much longer time period, or only increases tolerance within the bounds of the genetic ability of the species.

As mentioned in 6.5 the data suggested that in the non acidic environment, adaptation to Zn increased the tolerance of populations of *H. rivulare* to low pH and that pH tolerance increased with a greater resistance to Zn. This implies that part of the mechanism of tolerance in *H. rivulare* is common to both processes. The effect of increasing levels of Ca and Mg on the toxicity of Zn has been shown to improve the tolerance of *Hormidium* spp. to Zn (P. J. Say & B. A. Whitton, in press). However, similar increases in these and other elements (see 6.6) in the medium at the low pH values, did not cause a significant increase in growth of *H. rivulare*, whereas Ca did markedly reduce the toxicity of Zn at similar pH values. This indicates that the mechanisms of pH and Zn tolerance are not identical. It seems likely that the mechanisms involved in reducing the toxicity of Zn by increased levels of Ca is the same in both acidic and non acidic environments. The mechanisms involved in pH and heavy metal resistance are not known for the extreme acidic habitat. As mentioned above, the results suggest that an active mechanism is at least partly involved. There is some evidence to suggest that in bacteria H^+ is excluded by an energy dependent system (Manning & Cook, 1972). If such a system does operate, then it is likely that the enzymes involved are specialized and have a low pH optimum.

It seems unlikely that the pH of the cytoplasm alters drastically, otherwise the chlorophyll molecules would denature to form phaeophytin. Chlorophyll a analyses (5.8) did not indicate that such changes were taking place; it can therefore be assumed that either plastids are protected by a specialised acid tolerant membrane, or that H^+ are excluded from the cytoplasm. As mentioned in 1.73 a passive cation exchange system has been found to operate in Sphagnum (Clymo, 1963). The rate of exchange of ions was found to be proportional to the unesterfied polyuronic acids in the cell wall. It is not known whether algae also have these unesterfied acids present in their cell walls. It is also possible that an excess of negatively charged ions on the cell wall could produce an effective external barrier, by forming a layer of H^+ around the cell.

It is possible that both active and passive systems act simultaneously, with the passive system excluding the majority of ions and the active mechanism removing any excess H^+ that cross the cell wall. The possession of either one or both systems may account for the differences in tolerance shown by the species growing at low pH.

The observed increase in tolerance to heavy metals, with a decrease in pH, may be due to changes in the ionic state of the metal rather than the triggering of a metabolic process. At low pH, it is possible that ions form unusual ligands, which, because of their size, are unable to penetrate the cell wall. Alternatively, the low pH conditions may increase the availability of ions which are required in the mechanism responsible for heavy metal tolerance. In either case, preliminary accumulation studies on *Drepanocladus fluitans* suggest that heavy metal accumulation in the low pH environment is considerably reduced (E. J. H. Lloyd, pers. comm.)

Summary

An account is given of the water chemistry and photosynthetic flora of waters from 14 sites in England with a pH of 3.0 and below. In addition one stream (Brandon Pithouse Acid Stream, Site 3) with a pH gradient between pH 2.6 and >7.0 was studied in detail. The effect of pH on the growth of five species of algae was examined in the laboratory together with the effect of pH on the toxicity of Zn and Cu to *Hormidium rivulare* and *Euglena mutabilis*. As well as having a low pH the chemistry of the acid water was characterized by high levels of acidity, Fe, Al, Mn, Zn, Co, Ni, SO_4-S and Si. Levels of PO_4-P and combined inorganic N were moderately high, whilst the level of Pb was relatively low. The concentrations of many of the ions recorded were greater than those reported in the literature. Most of the cations mentioned above were significantly positively correlated at the 95% confidence level with pH and negatively correlated with acidity. However, pH and acidity were not always significantly correlated at this level.

Although the levels of the parameters measured at the source of Brandon Pithouse Acid Stream were relatively stable, the data collected from the surveys in late summer and late winter showed that at other sites there was seasonal variation. Many of the values were greater in the winter than summer period, even though dilution was greater in all streams during the winter. It is probable that the higher concentrations were

due to an increase in the water level which was in contact with the mineral bearing substrata in the mines.

The total flora of the water below pH 3.0 consisted of twenty four algae, two mosses and two flowering plants. Of these Euglena mutabilis, was the most widespread and abundant species. Pinnularia acoricola, Gloeochrysis turfosa, Nitzschia subcapitellata, Nitzschia elliptica var. alexandrina, Eunotia exigua, Chlamydomonas applanata var. acidophila and Hormidium rivulare were also commonly occurring and sometimes abundant.

Although the flora was restricted to a few species, individual organisms were often in very large numbers and produced macroscopically obvious growth. Maximum standing crop values for Brandon Pithouse Acid Stream were as large as those reported in the literature for other environments. dominated by a few species. There appeared to be little variation in the species composition of the streams, although there were temporal changes in the abundance of different species. This was reflected to some extent in the seasonal variation of the maximum standing crop values.

Although there did not appear to be any geographical distribution of species in England, comparisons of the species lists for different sites around the world suggest that the species are geographicaly distributed and that apart from a few species (eg. Euglena mutabilis and Eunotia exigua) the flora of the acid streams reflects to some extent the indigenous non acidic population.

Of the parameters measured pH seemed to have the greatest influence on the presence and absence of species. The number of species at a reach was significantly correlated at the 99% and 95% significance level for the Brandon Pithouse Acid Stream and the late summer survey. There was quite good agreement between the field and laboratory determined pH limits for growth. Many species were not recorded below pH 2.5 in field and *Hormidium rivulare* was restricted to pH values above 2.5 in the field and laboratory. The absence of Blue-green algae from the low pH environment supports the findings of other authors. It is also worth noting that the protonemal stage of the two moss species was considerably more widespread than the adult stages. It is suggested that those species which grow below pH 2.5 should be classed separately as extreme acidobiotic species.

Analysis of field data did not indicate that heavy metals were primarily responsible for restricting the presence of species in a stream. However, laboratory studies showed that high levels of Zn and Cu were toxic to populations of *H. rivulare* and *Euglena mutabilis* isolated from Brandon Pithouse Acid Stream. The toxicity of Zn and Cu increased markedly with an increase in pH and both organisms were most resistant at their optimum growth pH range. Cu was shown to be less toxic to *H. rivulare* than Zn at pH 3.5 but more toxic than Zn at pH 6.0. Ca was shown to antagonise the toxicity of Zn over a range of pH values, its presence did not effect the tolerance of the organism to pH. In view of the decrease in toxicity of Zn and Cu with low pH it is probable that these elements are most likely to restrict a species when it is growing near its pH growth limit.

Whilst it is likely that pH and possibly the synergistic effect of heavy metals, acidity and hydrogen ion concentration, limit the presence of species in the low pH environment, the observations suggest that physical factors such as iron hydroxide precipitate, current speed and substratum stability are important in controlling the standing crop of species in acid streams.

Marked morphological variations were observed in organisms growing near the pH growth limit. These were also noted in the laboratory in growth limiting concentrations of Zn. Eunotia exiqua, Pinnularia acoricola, Hormidium rivulare and Stichococcus bacillaris demonstrated the greatest variability in morphology.

Although considerably more work is necessary the results suggest that some species are pre-adapted to acid habitats but that some physiological adaptation takes place over a period of time. The mechanisms for pH tolerance are not understood. However, the results suggest that an active mechanism may be partially involved in pH and heavy metal resistance but that the systems are not identical.

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